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Chlorophyll Derivatives—*Their Chemistry, Commercial Preparation and Uses*

These products are commercially obtained in the United States to the extent of about 80,000 pounds annually by extraction of about 32,000,000 pounds of alfalfa, for the most part by only three concerns. They are finding increasing use, not only in the manufacture of popular deodorants but also in medicinal preparations for treating anemia and hypertension, as a healing agent and in oral hygiene.

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Introduction

Green plants are capable of synthesizing their complex constituents from the carbon dioxide of the atmosphere, water, and inorganic elements derived from the soil. The energy required for these chemical processes is supplied by solar radiation absorbed by the green coloring matter of the plants. This biochemical process of photosynthesis not only is responsible for our food supply, but, in the form of wood, coal and oil, it supplies three-fourths of our mechanical energy. It is significant, then, that in no instance has photosynthesis taken place in the complete absence of chlorophyll; even in plants of red or yellow coloration chlorophyll is present, its green color simply masked by the predominant pigment.

For the past 150 years chlorophyll and its role in plant life have been the subjects of extensive scientific investigation, and with the exception of a few minor points its chemistry has been well elucidated. Its mechanism within the plant cell, however, is still a mystery and probably will remain so as long as such investigations are confined to chlorophyll divorced from the living cell. However, with newer concepts aided by radioisotopes it should not be surprising to find this all important biochemical process solved within a relatively short time.

Chemistry and Isolation

Berzelius (6) was the first to attempt isolation of chlorophyll, and his work

paved the way for later investigators, most of whom employed such drastic methods that they succeeded in isolating only a mixture of chlorophyll decomposition products. Stokes (76) discovered spectroscopically that chlorophyll is a mixture of pigments, and he separated them by partition between immiscible solvents.

A relationship between the green pigment of plants and the red pigment of blood was postulated by Verdeil (81). This hypothesis was elaborated upon by Schunck (70), Marchlewski (48) and Hoppe-Seyler (37), and certain phases of such hypotheses are now known to be correct.

Due to its extreme sensitivity, ready solubility and the difficulty of separating it from contaminating substances, attempts to isolate chlorophyll were abandoned after the work of Hoppe-Seyler, and it remained for R. Willstaeter (86) and his students, working on an elaborate scale, to isolate and chemically identify the green plant pigments.

In general, plants do not form chlorophyll unless they are exposed to visible radiation. Etiolated plants contain a small quantity of yellow pigment and a minute amount of a green pigment which differs spectroscopically from chlorophyll (29). On irradiation of etiolated plants, chlorophyll is developed rapidly, and it has been suggested that a precursor of chlorophyll exists in plants grown in the dark. This substance, termed "proto-chlorophyll", has been isolated and structurally identified (29).

The coloring matter of plants is a mixture, and by suitable methods of extraction and partition there are obtained two green pigments (chlorophyll-*a* and chlorophyll-*b*), the orange to red carotenoids and the yellow to red xanthophylls. The ratio of chlorophyll-*a* to chlorophyll-*b* is usually 3 : 1; that of carotene to xanthophyll, 1 : 2; and the ratio of the total green to total yellow pigments is approximately 3 : 1. With the exception of certain aquatic plants these pigments are the same, irrespective of the plant tissue from which they are isolated.

The two chlorophyll components have the following composition: chlorophyll-*a*, $C_{55}H_{72}N_4O_5Mg$ (Fig. 1)*; chlorophyll-*b*, $C_{55}H_{70}N_4O_6Mg$ (Fig. 2). These parts owe their wax-like nature to the phytyl group in the molecule. Although both are tetra-pyrrole pigments, phytol and magnesium distinguish chlorophyll from hemin, the red pigment of the blood (Fig. 3). The latter does not contain an aliphatic molecule and magnesium is replaced with complexly bound iron.

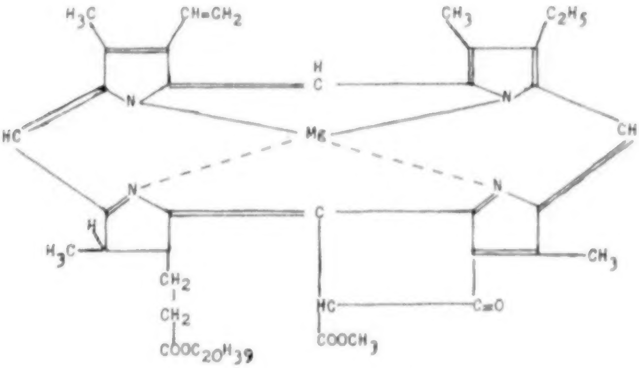
On drastic degradation, the two chlorophylls yield red crystallized compounds —porphyrins—with characteristic absorption spectra. They contain the tetra pyrrole nuclei linked with —CH= in the alpha positions and are substituted compounds; the nucleus carrying hydrogen atoms only has been named "porphine".

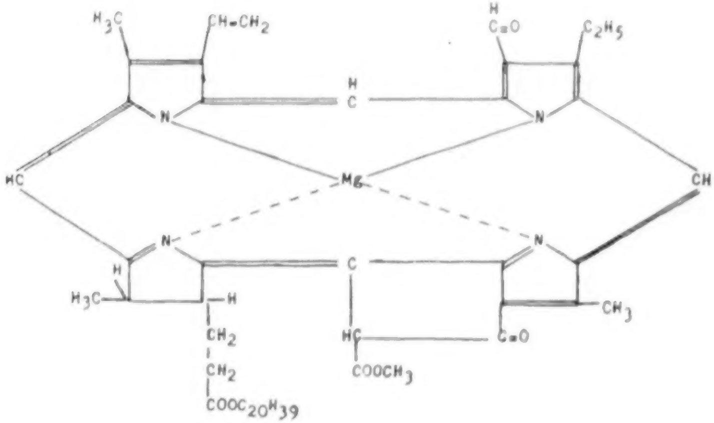
Two important modifications of the porphine (Fig. 4) system exist: the dihydroporphine or chlorin (Fig. 5) system and the phorbin (Fig. 6) system, differing from chlorin by addition of a carbocyclic ring.

Fischer (27) proposed a numbering system (Fig. 7) which differs considerably from that suggested by the International Union on Chemistry; because of its simplicity, it is preferred by chlorophyll chemists.

The magnesium complex salts of the porphyrins are called "phyllins". According to Fischer (27*a*), then, chlorophyll-*a* is the phyllin of 1,3,5,8-tetramethyl-4-ethyl-2-vinyl-9-oxo-10-carbomethoxy-phorbin-7-propionic acid phytyl ester, while chlorophyll-*b* is the phyllin of 1,5,8-trimethyl-4-ethyl-2-vinyl-3-formyl-9-oxo-10-carbomethoxy-phorbin-7-propionic acid phytyl ester. The two

* Throughout this paper only the chlorophyll derivatives obtained through degradation of chlorophyll-*a* will be illustrated.





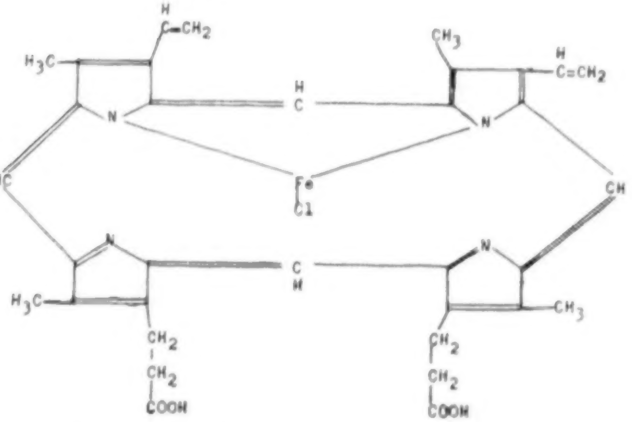
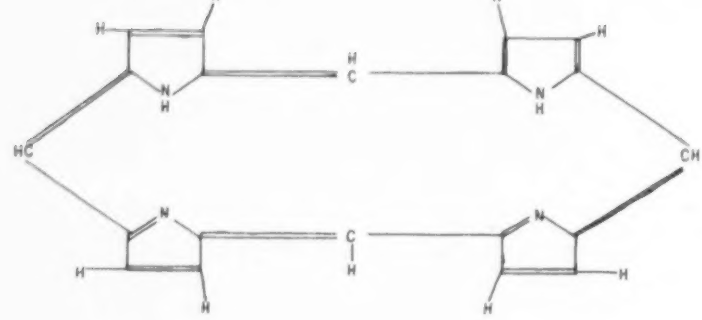
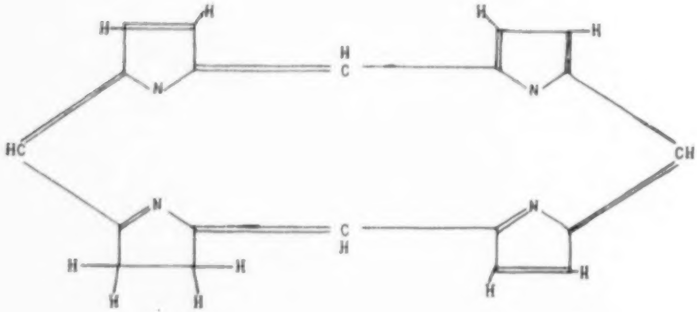


FIG. 1 (*Upper*). Chlorophyll-*a*.
FIG. 2 (*Middle*). Chlorophyll-*b*.
FIG. 3 (*Lower*). Hemin, the red pigment of hemoglobin.





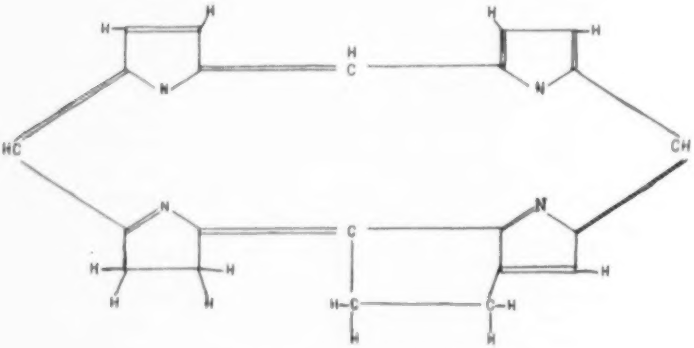
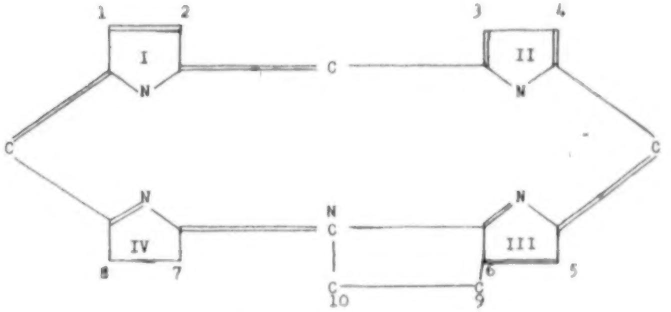


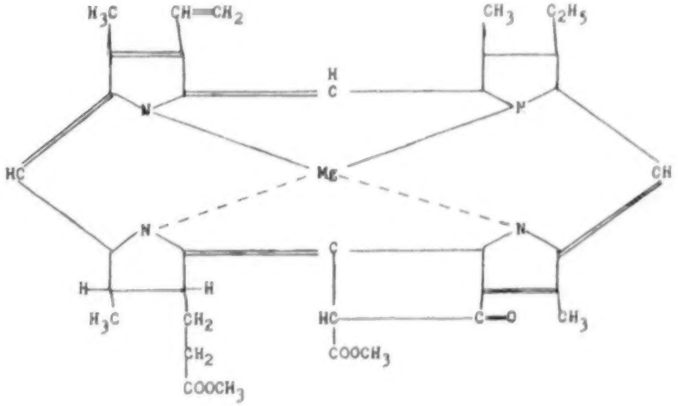
FIG. 4 (*Upper*). Porphine.
FIG. 5 (*Middle*). Chlorin.
FIG. 6 (*Lower*). Phorbin.

differ only in their substituents in position 3.

If a chlorophyll derivative is named together with a number as a suffix, such a number refers to the number of oxygen atoms in the molecule.

The enzyme chlorophyllase is the specific enzyme working on chlorophyll; by its action in the presence of alcohol, the phytyl group is replaced by the radical of the solvent. With methanol as solvent the methyl chlorophyllide dimethyl





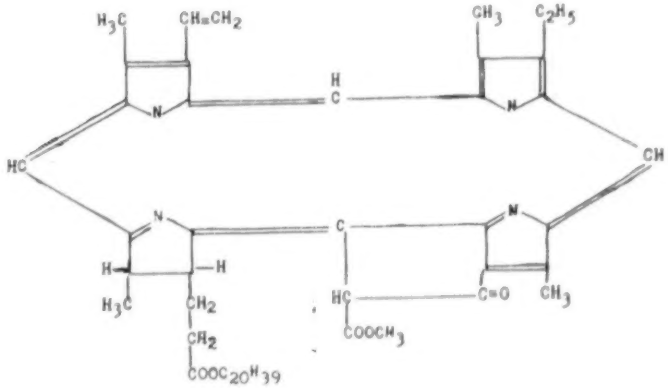


FIG. 7 (*Upper*). Numbering system proposed by Fischer.
FIG. 8 (*Middle*). Methyl-chlorophyllide.
FIG. 9 (*Lower*). Pheophytin.

ester is formed (Fig. 8). If the reaction is carried out in acetone the monocarbonic acid chlorophyllide is formed.

The phyllins of phorbins are very sensitive to acids, and even weak acids are capable of replacing the magnesium of the molecule with two hydrogen atoms. The two chlorophylls on treatment with acid yield pheophytins *a* and *b* (Fig. 9). With strong acids the phytyl group may also be removed. The products then are pheophorbides which still contain one carbomethoxy group.

Treating an ether solution of chlorophyll or a chlorophyll derivative having the carbocyclic ring intact with alkali causes opening of this ring, and depending on the conditions of reaction, numerous compounds are formed. Their characteristic system is the chlorin system (Fig. 10).

Propyl alcoholic potassium hydroxide not only cleaves the carbocyclic ring but causes in a secondary reaction an oxidation at carbon atom ten. The resulting compounds are still chlorins, but because of their characteristic absorption properties, they are grouped together as purpurins (Fig. 11) (**64**).

Willstaeter (**86**) found that a characteristic reaction of undamaged chlorophyll, pheophytin and pheophorbide in ether solution is that on addition of alkali the chlorophyll changes from green to yellow and back to green. Conant (**19**) found the first product of Willstaeter's so-called "phase test" for undamaged chlorophyll to be an unstable chlorin which on standing converts to pheopurpurin 18 (Fig. 14). Immediate esterification of the reaction mixture converts the unstable chlorin to pheopurpurin-7-trimethyl ester (Fig. 15). The phase test reaction was shown by Conant to be an oxidative hydrolysis with atmospheric oxygen acting as hydrogen acceptor. The hot saponification of either pheopurpurin 7 or its ester gives rise to a new chlorin, chlorin-f (Fig. 16). The unstable chlorin is a tricarboxylic acid or its lactone (Fig. 12 or 13).

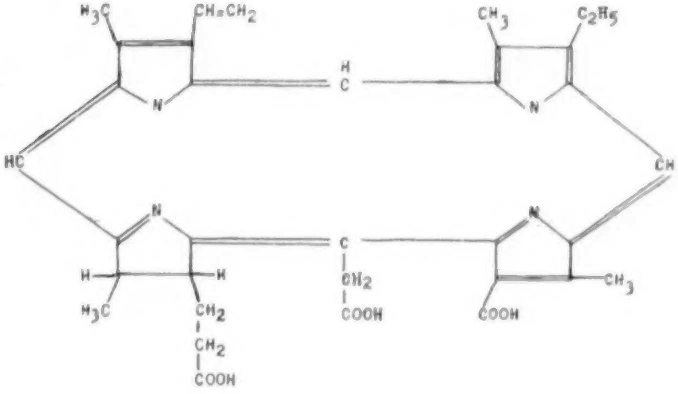
Willstaeter (**86**) also described a reaction of chlorophyll which he termed "allomerization". When an alcoholic solution of chlorophyll is allowed to stand for some time, the resulting "allomerized chlorophyll" is no longer capable of giving the phase test. From its alcoholic solution he prepared chlorin-g. Conant (**20**) showed that allomerized chlorophyll, on hydrolysis and removal of magnesium by acid and immediate esterification, gives the same product as does the phase test. This is in contrast to "unallomerized" chlorophyll which under identical treatment yields chlorin-e_6.

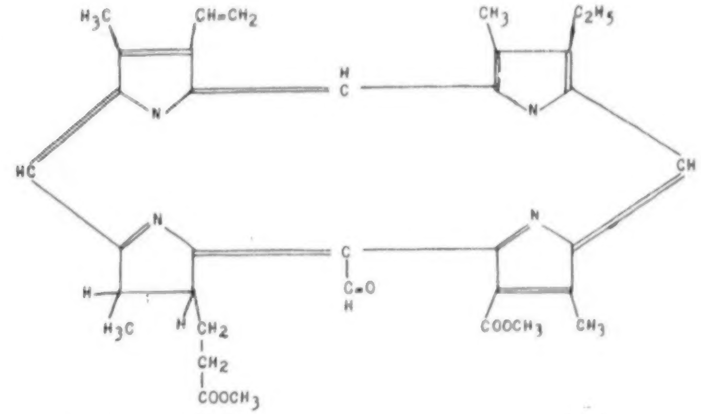
Catalytic hydrogenation of chlorophyll in acid medium differs according to the catalyst employed; palladium causes formation of colorless compounds with the addition of two moles of hydrogen. When platinum catalyst is employed, four moles of hydrogen are used. These leuco compounds may be reoxidized in air to the corresponding porphyrins. In alkaline medium those compounds having the vinyl group may also be reduced to leuco compounds.

The stability of the carboxyl groups in chlorophyll derivatives is variable. In some compounds decarboxylation takes place immediately on heating; in others the compound may lose carbon dioxide at temperatures well above the melting point.

Boiling pheophytin-*a* or a pheophorbide with methanolic potassium hydroxide for 30 seconds results in the formation of chlorin-e_6 (Fig. 17). This compound may be formed also by shaking an ether solution of purpurin 18 with methanolic potassium hydroxide. Chlorin-e_6 may be changed into chlorin-e_4 (Fig. 18) on boiling in pyridine.

In general, modern isolation of chlorophyll from plant tissue follows the procedures of either Willstaeter (**86**) or





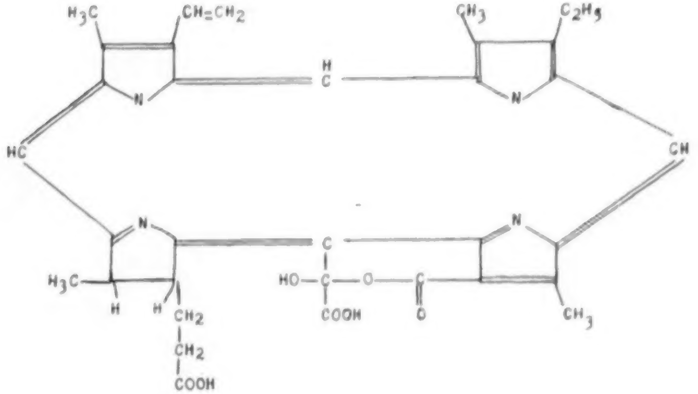
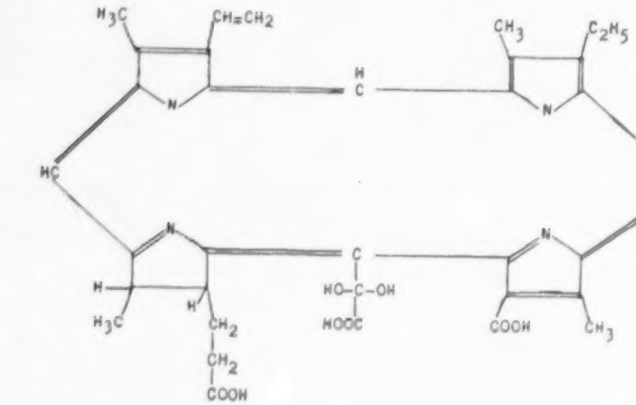
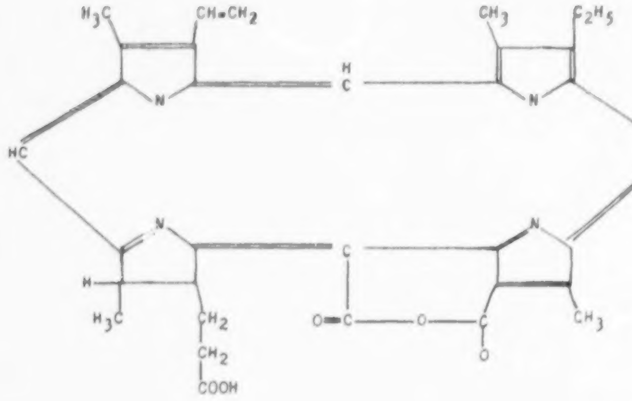


Fig. 10 (*Upper*). Chlorin-e_6.
Fig. 11 (*Middle*). Purpurin-5-dimethyl-ester.
Fig. 12 (*Lower*). Chlorin-g.





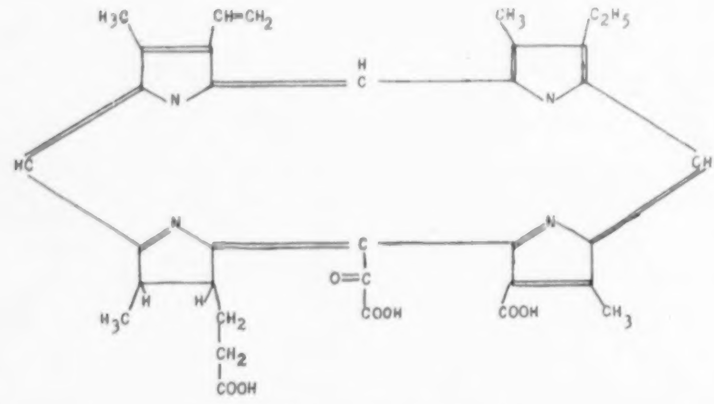
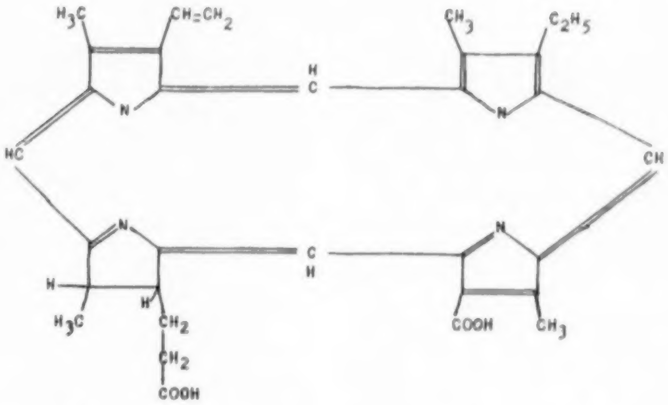
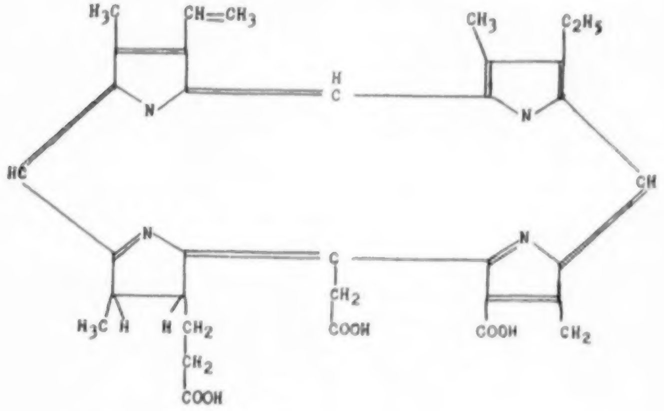


FIG. 13 (*Upper*). Unstable chlorin.
FIG. 14 (*Middle*). Pheopurpurin-18.
FIG. 15 (*Lower*). Pheopurpurin-7.





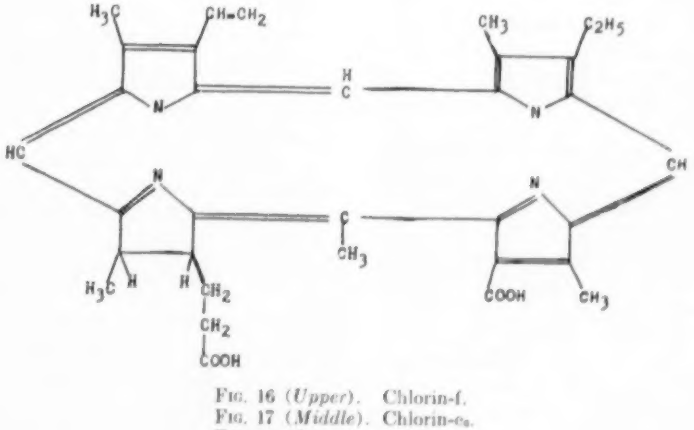


FIG. 16 (*Upper*). Chlorin-f.
FIG. 17 (*Middle*). Chlorin-e_6.
FIG. 18 (*Lower*). Chlorin-e_4.

Schertz (66). Willstaeter extracts dried plant tissue with aqueous acetone, transfers the pigments to petroleum ether, removes xanthophylls by washing the petroleum ether solution with 85% methanol, and frees the petroleum ether of methanol by exhaustive water washing, after which the chlorophyll precipitates and is removed by filtration through talc.

Schertz made a preliminary extraction of dried plant tissue with hexane for removal of carotene. A second extraction with 80% acetone-water is then carried out for removal of chlorophyll and xanthophyll. The pigments are transferred to hexane, and xanthophylls are removed by extraction of the hexane with 85% methanol. Finally the hexane is freed of methanol by exhaustive water washing, after which the chlorophyll precipitates and is recovered by centrifuging at 30,000 r.p.m.

Winterstein and Stein (87) obtain the pigment mixtures in a benzene-petroleum ether solution and chromatograph on powdered sugar. After development of the chromatogram, chlorophyll-*a* forms the lower blue green zone which is removed with acetone-ether solution. Chlorophyll-*b* forms the upper yellow-green zone of the chromatogram which may be eluted with ether. The principals of chromatography have been applied to commercial production of chlorophyll.

Experimental knowledge necessary for work on chlorophyll must be acquired by careful repetition and checking of definitely known reactions, and the chemistry of chlorophyll requires a number of special methods applicable with only minor changes to a large number of derivatives of chlorophyll. Their practical application is difficult because of the lability of the compounds, and success depends to a large extent on training and experience.

The spectroscopic behavior of chlorophyll pigments renders the spectroscope the most valuable instrument in chlorophyll chemistry.

Since in ether the absorption bands are very sharp, this solvent should be employed wherever possible. Spectra in alkali are often diffuse and hard to measure; in addition colloidal phenomena may cause considerable variation. Further, chlorophyll derivatives are extremely sensitive to light, and changes in absorption characteristics may occur while measurements are being made. Therefore examinations should be carried out in the minimum of time.

From the foregoing it will be determined that in the preparation of a chlorophyll substance one must be constantly aware of its extreme lability and sensitivity to light, acids and alkali, and that only slight changes in technique may cause fundamental changes in the character of the product undergoing preparation.

Factors Affecting Chlorophyll Content of Plants

The chlorophyll content of all plants is indeed variable. This variation may be attributed to one or more of the following:

Stem-Leaf Ratio. In leafy plants approximately 95% of the chlorophyll resides in the leaf, and in most commercial chlorophyll operations facilities are available for removing the stem fraction. The stem-leaf ratio may also be controlled by harvesting the plants at a stage of growth when the ratio is low.

In cereal and pasture grasses the chlorophyll content is also higher at an early stage of growth but declines rapidly as the plant matures.

Fertilization. It has been demonstrated (24) that plants deprived of iron do not normally produce chlorophyll. The chemical form in which the iron is presented to the plant seems to make

little difference so long as the pH is kept such that there is a soluble iron compound available at all times.

Maiwald (47) found that the amount of potassium salts used as fertilizer greatly influenced the intensity of greening in leaves.

McHargue (50) observed that wheat seedlings grown in manganese-free medium grow normally for the first few weeks, but after this time the development of chlorophyll is retarded and the plants become yellow.

Kostychev (43) demonstrated that an excess of certain minerals in the soil may cause diminution of the chlorophyll content. He showed that plants which had become accustomed to soils poor in lime displayed chlorosis when grown on soils which had been heavily irrigated with lime water.

An inadequate supply of potassium may cause chlorosis, but an excessive quantity of this element may have the same effect if the supply of nitrogen is not large enough (31).

Magnesium is present in the chlorophyll molecule, and its complete absence inevitably leads to chlorosis. Fleischer (28) and Kennedy (41) found that much more magnesium is required to bring about the full rate of photosynthesis than to prevent chlorosis.

Manganese, like iron, in some way seems to be essential in the production of chlorophyll. In its absence a characteristic mottled chlorosis develops in the younger leaves.

Water. Desiccation of leaf tissues not only inhibits synthesis of chlorophyll but sems to accelerate disintegration of the chlorophyll already present. A familiar example of this effect is the browning of grass during droughts.

Light. Formation of chlorophyll depends not only on the intensity of light but also on its spectral composition. Johnston (40) found less chlorophyll in plants grown in red light, while Stoklasa (77) noted that formation of chlorophyll occurs more rapidly in ultraviolet light.

Willstaeter (86) observed that leaves grown in shade are much richer in chlorophyll than those grown in full sunlight.

Chlorophyll Production

Although chlorophyll has been employed in the coloring of soaps, waxes, etc., for many years, its recent application to medicinal, deodorant and industrial uses has resulted in its manufacture on a scale far exceeding its earlier production. The usage of chlorophyll has expanded until there are now three major producers; The American Chlorophyll Company, Lake Worth, Florida; The Keystone Chemurgic Corporation, Nazareth, Pennsylvania; and The National Chlorophyll and Chemical Company, Lamar, Colorado. When operating at capacity the combined production of these companies is estimated to be in the neighborhood of 80,000 pounds of chlorophyll derivatives per year which in turn requires approximately 32,000,000 pounds of raw material annually.

During the period when chlorophyll products were being introduced to the public, several other chlorophyll companies were formed, but as a result of production and other difficulties, most of these have discontinued operations.

Raw Material. Except for a few more or less experimental runs, dehydrated alfalfa has served exclusively as the raw material for chlorophyll production. Although other plants are known to be substantially richer in chlorophyll, none is cultivated and artificially dried on the same extensive scale as alfalfa. Therefore, dehydrated alfalfa is a readily available and economical source of chlorophyll, and the rapid and successful expansion of the chlorophyll industry may be attributed to the alfalfa dehydration industry. In general, if alfalfa is harvested at an early stage of growth and dehydrated immediately, one should ex-

pect an excellent yield of chlorophyll. Although during dehydration chlorophyll degradation occurs, such degradation, as will be subsequently shown, may be desirable.

The usual chlorophyll production process consists of the following steps: *a*) primary extraction, *b*) separation of contaminating substances, *c*) conversion to stable derivatives.

Primary Extraction. This first step is accomplished by either batch or continuous processes, and a wide variety of solvents are available for the purpose. In some instances a mixture of solvents is employed, but, because of the economics involved in the fractionation of such mixtures, mixing of solvents should be avoided wherever possible. Although no quantitative data are given, the Oil, Paint and Drug Reporter (**56**) lists some 58 solvents for chlorophyll extraction; among them are:

Ethyl alcohol
Ethyl acetate
Diethylene glycol
Diethylene oxide
Ethylene chloride
Ethylene glycol
Diethyl ether
Diethylene glycol monoacetate
Diethylene glycol butyl ether
Dibutyl phthalate
Diacetone
Cyclohexanone
Chloroform
Cellosolve
Carbon tetrachloride
Carbon disulfide
Amyl alcohol
Acetone
Glycol ether acetate
Isopropyl alcohol
Propylene oxide
Toluene
Trichloroethylene
Paraffin oil
Isopropyl ether
Ethylene glycol butyl ether
Ethylene glycol iso butyl ether
Ethylene glycol diethyl ether
Ethylene glycol isoamyl ether
Ethylene glycol propyl ether
Ethylene glycol methyl ether
Dichloroethylene
Carbinol
Butyl cellosolve
Butyl butyrate
Butyl alcohol
Butyl acetate
Amyl alcohol
Butyl acetate
Acetylene tetrachloride
Glycol diacetate
Glycol monoacetate
Methyl cyclohexanone
Tetraethylene glycol
Benzene
Perchloroethane
Fats
Fixed Oils

Although numerous solvents are available for the extraction of chlorophyll, because of the complete and rapid extraction obtained most chlorophyll producers prefer aqueous-solvent mixtures. Willstaeter (**86**) gives the following data on the degree of extraction obtained with aqueous solvents:

EFFICACY OF AQUEOUS SOLVENTS ON CHLOROPHYLL EXTRACTION

Volume percent	Gm. chlorophyll extracted		
	Acetone	Ethyl alcohol	Methyl alcohol
100	3.05	5.4	6.2
97	7.70	..	..
95	...	6.6	2.9
90	9.45	8.5	..
85	9.75	7.5	0.4
80	9.15	6.8	..
75	7.80	4.2	..
70	7.10	..	..
65	5.60	..	..

In some instances the dehydrated alfalfa is rehydrated to about 15 percent moisture content, the net result being the same as extraction with aqueous solvents.

Crude Extract. Because of the extreme lability of chlorophyll, the solvent is usually recovered from the crude extract at reduced pressures and temperatures.

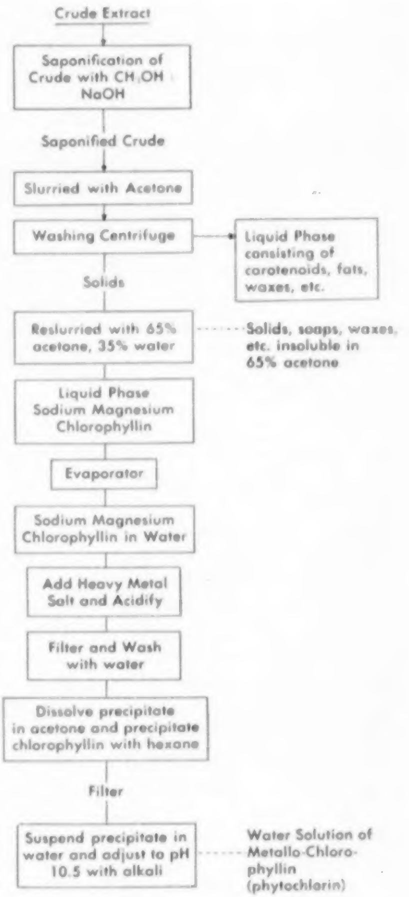
With a non-aqueous solvent, such as acetone or isopropanol, the crude extract after removal of solvent has the following approximate composition:

Chlorophyll	6.0–8.0%
Carotene	0.2–0.4
Xanthophyll	0.3–0.5
Waxes	?
Sterols	1.0–2.0
Oils	?
Tocopherol	0.1–0.2
Vitamin K	?
Phytol	2.0–3.0

When an aqueous-acetone solvent is employed, the quantity of lipoidal substances extracted is reduced considerably, but the extract contains considerable quantities of water-soluble substances which must be removed before purification of the chlorophyll in the crude can be accomplished.

Separation of Contaminating Substances. The nature of the crude chlorophyll extract varies according to the solubility of the leaf constituents in the particular solvent used, and the techniques of separation and purification of the chlorophyll must be varied according to the solvent. For example, if aqueous solvents are employed, the water-soluble substances must be removed by transferring the crude extract to a water-immiscible solvent; these materials may then be removed by washing with water, while the lipoidal substances remain in the non-aqueous phase. On the other hand, if non-aqueous solvents are employed, considerably more lipoids are extracted. While some of these may be removed by saponification, the remainder must be removed by de-waxing or fractionation between solvents.

The following flow description gives a procedure for purifying chlorophyll from a crude extract obtained through the extraction of dehydrated alfalfa meal containing 15 percent moisture with a solvent consisting of equal parts of acetone and hexane.



Flow-diagram of a procedure for purifying chlorophyll from a crude acetone-hexane extract of alfalfa.

Conversion to Chlorophyllin. The crude extract is reacted with methanolic sodium hydroxide. By this treatment the phytyl radical of the chlorophyll is replaced with sodium as are the alkyl radicals of the carbomethoxy groups. The water-soluble sodium magnesium chlorophyllin thus formed is simply the magnesium complex of the phytochlorins (86).

When saponification is complete, the mass, which consists principally of chlorophyllins, carotenes, xanthophylls, waxes and sterols, is slurried with acetone for extraction of the unsaponifiable substances. The slurry is passed through a washing centrifuge; the liquid phase is taken to an evaporator for solvent recovery. The chlorophyll-containing solids phase is reslurried with aqueous acetone (35-65), in which the chlorophyllin is soluble, and passed through another washing centrifuge. Here the liquid phase containing the chlorophyllin is passed through an evaporator for acetone recovery, the product being a water solution of sodium magnesium chlorophyllin. The solids phase, which consists principally of waxes and soaps, is discarded.

Conversion to Stable Derivatives. Those chlorophyll compounds containing complexly bound magnesium are the

most unstable of all chlorophyll derivatives, and for the purpose of stabilization a heavy metal must replace the magnesium of the chlorophyll nucleus. Such replacement is easily accomplished, the reaction medium differing according to the metal to be introduced. For preparation of the copper, cobalt, iron, nickel, silver, zinc, manganese or gold complexes, acid solution is required. In weakly alkaline solution the mercury, tin, lead or thallium complexes are formed, whereas the sodium, potassium, cesium, rubidium and magnesium complexes are formed only under strongly alkaline conditions.

While up to the present time the copper chlorophyll complexes have been in most general usage, more recent applications require a heavy metal other than copper, and in some instances a water-soluble compound, in which hydrogen replaces the metallic nucleus, is used.

From the water solution of sodium magnesium chlorophyllin, prepared as above, the exchange of magnesium for heavy metal is readily accomplished by addition of an excess of a copper or other metallic salt to the hot chlorophyllin solution, followed by acidification. The magnesium is replaced with copper, and the sodium ions are removed from the molecule, thus precipitating the water-insoluble copper phytochlorin. The copper chlorin is contaminated with a large quantity of fatty acids which are formed from the soaps which accompany the sodium magnesium chlorophyllin, and these must be removed by washing the precipitated phytochlorin with a solvent in which the fatty acids are soluble but the copper phytochlorin is insoluble, or, the metallochlorin derivative and fatty acids may be dissolved in acetone and precipitated therefrom with hexane; in this instance the fatty acids remain in the hexane.

After filtration of the precipitated chlorophyll the blue-black powder is suspended in water and the hydrogen of the carboxyl groups is replaced by addition of an alkali metal. On the addition of alkali, the salt of copper phytochlorin is formed, thus becoming water-soluble and dissolving. The resulting solution of sodium or potassium copper phytochlorin may be used as such or dried to a powder of 90 to 100 percent purity.

Chlorophyll Derivatives of Commerce. Willstaeter (86) demonstrated that, depending upon the purity of the initial chlorophyll and other conditions, the reaction of chlorophyll with alkali could lead to either a single tricarboxylic acid (phytochlorin-e) or a mixture of mono-, di- and tri-carboxylic acids. For example, when pure chlorophyll was subjected to rapid and hot saponification, the isosodium magnesium chlorophyllin was obtained; acidification of this compound yielded the tricarboxylic acid, phytochlorin-e. However, if cold saponification was used, the same procedure led only to a mixture of mono-, di- and tricarboxylic acids, e.g., chlorin-g, chlorin-f, chlorin-e and perhaps other degradation products.

Furthermore, Willstaeter (86) showed that the same procedure when applied to pheophytin or allomerized chlorophyll, regardless of the conditions of saponification, led only to a mixture of the feebly basic chlorins-f and -g, with lesser quantities of chlorin-e present.

Unfortunately, under the best of commercial processes the dehydration of plant tissue results in the decomposition of at least a part of the chlorophyll, and under extremely adverse circumstances total degradation of chlorophyll may take place; liberated plant acids and high temperatures result in the formation of pheophytin, allomerization and isomerization of the chlorophyll. Since it is precisely these chlorophyll degradation products which result in the formation of the feebly basic chlorins-f and -g, along with lesser amounts of phyto-

chlorin-e, from dehydrated plant tissue one can expect no more than a mixture of the chlorophyll mono-, di- and tricarboxylic acids. Therefore, the products of commerce are truly the sodium or potassium salts of a mixture of copper phytochlorins-f, -g and -e, and in some instances, the purpurins. Except for the replacement of magnesium with a heavy metal, these compounds are identical with the chlorophyllins.

Uses

Industrial

Photochemical Sensitizer. The first indication of an industrial use for chlorophyll was given by Becquerel (5, 57) who employed chlorophyll as a sensitizer of collodion and silver bromide or iodide film. Several chemical reactions can be carried out with chlorophyll in this capacity; the photochemical reduction of methyl red by phenylhydrazine in the presence of chlorophyll has been reported by Ghosh and Sen Gupta (32). Bohi (9) brought about the photochemical reduction of a number of dyestuffs belonging to very different classes of chemical compounds.

Coating and Protective Agent. The ability of the chlorophyll molecule to build up laminar films with either hydrophilic or hydrophobic properties is well known. Although the physics of such laminar films and monomolecular layers is a relatively new field, the research reported to date has already disclosed a wide and potential field of commercial application of them, both organic and inorganic (52). By using such films, special optical properties, e.g., non-reflectivity and multilayer structure, can be achieved (3), and protective coatings for metals and for use in food preservation are possible.

Among such commercial applications for chlorophyll could be the coating of the inside of sealed bags and other container packages in order to absorb oxygen or other gases. In agriculture, seedlings can be coated with a thin multilaminar layer which, if of a chlorophyll nature, could conceivably carry various micro-nutrients and even growth hormones. Sugar beet seedlings, chicory and carrot seedlings have been treated in this way, in order to improve rate of germination and to enhance root fixation.

Food-Coloring and Micro-Nutrient-Carrying Agent. During the past few years increasing attention has been directed in the food industry to the improvement of the appearance of food products, as offered for sale on the retailer's shelf. This attention has extended to the marketing of fresh vegetables and the problem of giving them a fresher and more pleasant appearance over a longer period of time. One example in this regard is lettuce which tends to assume a weak green or white appearance long before its food value has in any way been impaired. Loss of salability inevitably is the result.

It would obviously be desirable to improve and preserve the natural green color by use of additive coloring matters. Aside from the fact that many green dyes now available for this purpose are obviously unsuitable because of their toxicity, even those which are non-toxic do not have the natural color of the green leaf which is required. Chlorophyll or its derivatives would be the obvious coloring agent to use in this respect. These compounds are not only the natural color of the plant itself, but are also non-toxic, even in high concentrations. In addition, if properly applied, such derivatives may actually improve the food value of the vegetable itself. For example, cobalt or iron chlorophyllin used as a dyestuff could definitely enhance the hematopoietic properties of lettuce, so that this vegetable could rival the present well known spinach and similar greens in this respect.

Other minor applications of chlorophyll compounds in food production

would include the use of chlorophyll chelates as carriers of micro-nutrients like cobalt, zinc, manganese, iron and molybdenum, either in gardening or in hydroponics. The advantage of chlorophyll compounds in contrast to other carriers lies in the fact that chlorophyll chelates would deliver metal atoms to plants much more slowly, thus preventing "burning" of them.

The same compounds could be used for treatment of chlorosis in orchards, or in gardening.

Chelating Agent. It is well known that complex molecules which have a strong resonating molecular structure have also strong chelating power which is stable down to a very low pH range. Outstanding examples of this contribution of a strong resonance effect to chelate stability are the natural pigments, such as chlorophyll or heme, in which a porphyrin derivative is bonded to bivalent metal.

The extraordinary stability of these substances is indicated by the fact that chelates of copper and other bivalent metals with porphyrin derivatives are not decomposed by strong mineral acids.

In the chlorophyll molecule and its derivatives the resonating power of the chelate can be varied according to the number and nature of the side chains involved The absorption constant as well as the distribution coefficient of the chelate can thus be changed, which makes these compounds very interesting for application in ion separation and solvent extraction, both of which are now being applied increasingly to rare earth separations, as well as in nuclear chemistry and other industrial fields.

Catalytic Agent. The special structure of the chlorophyll molecule confers upon this pigment the ability to exhibit activity as a catalyst. Its function in photosynthesis is probably the largest and most effective application in this regard known to date. Although this important function has long been recognized, the mechanism of photosynthesis and the role of chlorophyll therein have not been elucidated. The problem has, however, become an extensive field of research all of its own. The lack of data on the catalytic action of chlorophyll compounds is undoubtedly a result of their great sensitivity and their recent high cost.

Chlorophyll can act, in general, as an oxidation-reduction catalyst. Such reactions can be purely chemical or photosensitized. For example, a photosensitized reaction with molecular oxygen would follow a general scheme like this:

$$O_2 + \text{Chloroph} \rightarrow O_2 - \text{Chloroph}$$

$$O_2 - \text{Chloroph} + A \rightarrow \text{Chloroph} -O-O-A \rightarrow \text{Chloroph} + AO_2$$

Such reactions could be used, for example, for oxidation of organic compounds. Schmidt (67) used chlorophyll as a catalyst in preparing carboxylic acids from reducing sugars. The action of chlorophyll as an anti-knock agent in fuel combustion is undoubtedly based upon such a reaction, as is also the use of chlorophyll in the tobacco industry (35) to decrease the amount of carbon monoxide formation in cigarette smoke.

Chlorophyll can also act as a hydrogen donor in catalysts. Vitamin C can be reduced in this way, in the presence of cobalt-dehydrogenase, under the action of light (44).

The influence of the chelated metals with chlorophyll is similar to the usual strong activating influence of iron or cobalt in catalysts, e.g., in the photooxidation of methyl linoleate (38) as an anti-oxidant in fats (17).

Sun Screening Agent. The degree of protection afforded against sunlight or other polychromatic radiation by a sunburn preventive is a function of the spectral distribution of the radiation, the transmission spectrum of the preventive,

and numerous factors affecting the threshold of the individual.

During exposure of the skin to sunlight an immediate reddening is usually observed. This is a heat erythema provoked by the long visible rays and disappears shortly after radiation is stopped. The erythema resulting from shorter ultraviolet rays of sunlight, 3000 Å and below, is produced only after a latent period of from one to ten hours (23). If the dosage of ultraviolet light is large there may be desquamation of superficial skin layers or even blistering or more severe damage (23). Pigmentation or sun tan on exposure to sunlight is due to the longer ultraviolet rays of 3200 Å and above. Protection against sunburn is provided by any material which absorbs ultraviolet light of wave lengths shorter than 3200 Å. This is accomplished by window glass, and it is possible to gain the same result by applying to the skin a substance which filters out the responsible radiation (8).

Many suntan preparations have been devised and marketed, but relatively few are effective and some are entirely useless (8). The unique light absorption properties of chlorophyll and its derivatives causes them to be a valuable ingredient of sun tan preparations, for, while absorbing the harmful shorter ultraviolet rays, the pigment-forming longer ultraviolet rays are allowed to come in contact with the skin.

Wunderer (88) evaluated many substances for the protection of human skin against strong radiation. He observed that most preparations designed to protect against erythema do not eliminate the active wave lengths. Of the newer materials studied, chlorophyll was capable of absorbing the harmful rays.

Vidal (82) studied the screening efficiency of several compounds against sunburning of human skin; neither nicotinic acid nor resorcin absorbs ultraviolet light when applied to the skin, but they were useful as internal protectants against sensitization. Tannin and quinine were likewise ineffective, but chlorophyll in external application efficiently protected against sunburn.

Roffo (62) successfully used two methods for protection of the skin against actinic radiations: *a*) by obtaining pigmentation of the skin by means of controlled sun baths of a duration previously determined by graduated exposure; *b*) by covering the skin with creams containing a substance having a selective absorption of the ultraviolet rays. The substance used by Roffo was chlorophyll, and he noted that the vehicle, which in his case was either gelatin, glycerol or lanolin, was also of importance.

Schreiber (68) described the skin protective substances issued by the quartermaster corps. Because the skin preparations had to be suitable for use under any climatic condition, three color combinations were developed: *a*) green on loam for white soldiers in non-snow environment; *b*) green and sand for negro soldiers in non-snow environment; *c*) white and loam for troops in snow environment. This work suggests military use of chlorophyll.

Tinao and Vidal (78) found that rubrophrene, fluorescein, eosin and acridine derivatives were of high ultraviolet-absorbing power. In view of the frequency of sensitization caused by these substances it was concluded that the same agents which sensitize within the body protect most efficiently when applied to the skin. Sensitization by certain chlorophyll porphyrins has been demonstrated (69, 45), and this supports in theory the work of Roffo, Wunderer and perhaps others who have found chlorophyll derivatives to be an effective sun-screening agent.

Others. In addition to the foregoing, the oxidation velocity of some ethylenic double bonds is increased in the presence

of chlorophyll (60), use of chlorophyll as accelerator in the vulcanization of rubber has been patented (12), photochemical decomposition of sugar is prevented by chlorophyll (7), the pigment is one of the best dispersants for carbon black printing inks but uneconomical for such use at present (30), a patent relating to the use of chlorophyll compounds in camouflage has been assigned to the U. S. Navy (80), chlorophyll when added to drying oils accelerates the drying process in direct proportion to the amount of chlorophyll present (65), a patent has been granted on the use of heavy metal chlorophyll derivatives as antiknock compounds for gasoline (34), and, finally, some chlorophyll derivatives are easily incorporated into plastics, to which they impart a remarkably stable green coloration.

Medical

Anemia. The establishment of the chemical relationship between the blood pigment and that of green plants was responsible for the first investigations of the pharmacologic applications of chlorophyll. Dr. Emil Burgi (14), Director of the Pharmacological Institute of the University of Berne, Switzerland, was among the first to produce evidence for the therapeutic value of chlorophyll and its derivatives. Initially Burgi found that when rabbits, rendered anemic by artificial means, were left without medication, their blood was renewed within six to eight weeks; addition of chlorophyll or iron shortened the regeneration by two weeks. But when chlorophyll was used in combination with iron, the normal blood count was restored in eight to ten days. Recovery in these experiments was judged by reference to the hemoglobin content and erythrocyte count. This experiment was performed four times, always with the same result. Burgi (14) also treated 126 anemic patients with a chlorophyll-iron compound. These cases included 39 of pure chlorosis; the others were regarded as cases of secondary anemia developed against a background of disease or else a nutritive deficiency. The patients ranged in age from 18 to 52 years and were of both sexes. The medication, consisting of chlorophyllin in combination with iron, was given orally. The efficacy of the medication was determined by hemoglobin and blood count. In all instances the benefit of the treatment was apparent not only in the blood picture but also by increased appetite and general condition of the patient.

Dr. Arthur J. Patek (58), Harvard Medical School, studied the effect of chlorophyll on the regeneration of blood. In two series of observations on the same patient, pheophytin was fed by mouth, averaging one gram daily for seven days, without effect on the red blood cell count, the concentration of hemoglobin or the reticulocyte count. Negative results were noted also in two patients fed sodium chlorophyllin daily, in amounts averaging one and one-half grams daily for seven days. In one case five ml. of chlorin-e was injected intramuscularly daily for five days without effect. In three series of observations on two patients a trial period of treatment with ferric ammonium citrate in daily doses of 0.6, 0.6 and 1.8 grams, respectively, was given for ten days. In the first case a moderate response of reticulocytes occurred; in the other two, only a slight response took place. With this medication continued, crude chlorophyll was added in amounts of three, four and five grams, respectively, for ten days, but an additional effect was not observed. Thus very small doses of iron with relatively large doses of chlorophyll were no more effective than iron alone.

In seven series of studies on five patients ferric ammonium citrate in suboptimal doses was given daily for at least ten days, during which the usual

response of reticulocytes occurred with the peak between the fifth and eighth day. Iron medication was continued and the chlorophyll product added in daily amounts comparable by weight to the dose of ferric ammonium citrate. In all instances a second reticulocyte response occurred with the peak on about the fifth day. There were three trials with crude chlorophyll, two with pheophytin and two with sodium chlorophyllin. During the first period of ten days with iron alone, the average gain in concentration of hemoglobin was 4.6 percent, and during the second period, 7.8 percent. These figures allow only a rough estimate of the gain in concentration. In short intervals there may be a lag of response so that one period overlaps the second. However, it appears that the rate of increase in concentration of hemoglobin with iron and chlorophyll therapy exceeds that with iron alone.

In a series of studies on two patients, 0.1 gram of iron citrate was injected intramuscularly daily for ten days. Moderate response of the reticulocytes occurred. With identical therapy continued, sodium chlorophyllin was fed by mouth; to one patient 0.7 gram was administered daily for eight days, and to the other one gram daily for six days. A second reticulocyte response took place promptly, which indicated that the combined effect was more potent than that of iron alone. In a series of three studies on two patients, iron citrate in doses of 0.1 gram daily was injected intramuscularly for ten days. A small reticulocyte response occurred. With this therapy continued, chlorin-e was injected daily in doses of five cc. for five days. Each injection corresponded to 0.1 gram of the solid material. A second sharp rise in the reticulocyte count occurred in both instances.

Patek commented as follows: "Several possible causes may be considered to explain the ineffectiveness of chlorophyll derivatives alone. In certain cases of anemia there may exist a state of pure deficiency of iron: there may be no want of pyrrol or of pigment substances, and in such instances one would expect no benefit from chlorophyll therapy. Since the materials in crude chlorophyll and pheophytin are relatively insoluble, there would arise also the question of embarrassment of absorption from the gastro-intestinal tract. However, the soluble chlorophyllin salt administered orally and chlorin-e administered parenterally likewise failed to affect the blood picture when given without iron. It is apparently essential, therefore, that iron be present before a chloropylll substance can be utilized. Iron salts are involved in the formation of chlorophyll, although they do not enter into its molecular structure. Emerson and Noack showed that the growth of chlorophyll in plants can be controlled by the presence of iron salts. An analogous catalytic effect perhaps takes part in the formation of hemoglobin.

It is known that chlorophyll combines chemically with iron in the ratio of about ten molecules to one. In those cases in which there was a response to therapy this ratio was never exceeded, the highest being ten to one. In the cases in which there was no response, with a large excess of chlorophyll, the ratio to iron was as high as 35 to one. It may be that in the latter, iron was fixed or buried because of its strong affinity for chlorophyll and thus became unavailable for absorption from the gastro-intestinal tract.

In those cases in which a second reticulocyte response occurred, together with an increased rate of regeneration of hemoglobin following the administration of chlorophyll derivatives and iron, the response appears to have been significant. A reticulocyte response occurs, to be sure, as the result of hemolysis, but signs of this were not observed. The

significant second responses took place on the addition to iron medication of chlorophyll, sodium chlorophyllin, pheophytin and chlorin-e In the latter two substances the magnesium radical is missing In all preparations iron was present in insignificant traces. Hence it is illogical to consider that a mineral contamination of iron or magnesium is responsible for the increased hematopoiesis observed. The fact that similar phenomena occurred when material was administered parenterally excludes a change of gastro-intestinal absorption as the conditioning factor when material was given orally.

In patients given chlorophyll and its derivatives, there is a rhythmic pattern of hematologic response. The time of onset of the response and the course taken by the reticulocyte count, the increase of concentration of hemoglobin and the improved sense of well-being were similar in all patients in whom a response occurred. For these reasons it seems likely that the changes observed were not accidental and that the substances used provided a special hematopoietic stimulus. Whether they acted in the intermediary stage of iron metabolism, that is, utilization of iron, or whether they acted simply in a substitutive way, was not ascertained ".

Patek summarized as follows:

a) Twenty series of observations on 15 cases of chronic hypochromic anemia were made with the aim of determining what effect the administration of chlorophyll products might produce on regeneration of the blood.

b) In five instances the administration of chlorophyll and its degradation products alone did not produce changes in the blood.

c) In three instances the administration of very large doses of crude chlorophyll with very small doses of iron likewise did not produce change in the blood.

d) In twelve sets of observations on nine cases the administration of chlorophyll products after a period of medication with comparable amounts of iron was followed by an orderly and significant increase of the concentration of the hemoglobin in the second reticulocyte response. This combined effect was produced when materials were given parenterally as well as orally. The studies suggested that the body can use preformed pyrrol substances for the building of hemoglobin.

In **1936** Hughes and Latner (**39**) published a paper entitled " Chlorophyll and Hemoglobin Regeneration after Hemorrhage ". In their work, rabbits were rendered anemic by repeated bleeding from an ear vein and then allowed to recover. During their recovery period the test animals were fed with chlorophyll dissolved in olive oil. Tests were made with varying doses of pure chlorophyll and with a large dose of crude chlorophyll, and one with a magnesium-free chlorophyll derivative. Pure chlorophyll in large doses had no effect on the speed of hemoglobin regeneration after hemorrhage, whereas very small doses markedly increased the speed. Crude chlorophyll is effective in large doses. The magnesium-free derivative also aids regeneration when given in large doses.

In **1932** Aoki (**2**) published a paper entitled " Experimental Studies on the Effect of Chlorophyll upon the Formation of Hemoglobin ". He summarized as follows:

a) Injection of chlorophyll into the normal rabbit produces no change in the number of red corpuscles or in the amount of hemoglobin.

b) In rabbits made anemic by injection of phenylhydrazine hydrochloride the injection (intravenous) of water-soluble chlorophyllins resulted in a marked increase in hemoglobin.

Aoki believed the latter effect attributable to the pyrrole ring in the chlorophyll molecule.

While no information as to their

mechanism is available, the foregoing works demonstrate the value of chlorophyll and chlorophyll derivatives in the treatment of anemia.

Bacteriostatic Agent. In May, 1944, Smith (74) reported on the anti-bacterial properties of water-soluble chlorophyll derivatives. As a result of these investigations, which were supported by a grant from the Committee on Therapeutic Research of the Council of Pharmacy of the American Medical Association, he was able to state that evidence had been accumulated to support the premise that chlorophyll acts to produce an environment unfavorable to the growth of bacteria instead of by any direct action upon the organisms themselves. The ability of these derivatives to hold back anaerobic bacterial growth points, in his opinion, to an oxidation mechanism. In brief, chlorophyll is not strictly bactericidal but does exert a definite bacteriostatic action and may even display a bactericidal effect against certain organisms under suitable conditions.

Healing Agent. In the foregoing paper Smith suggested that prompt tissue repair response to chlorophyll therapy might be the chief factor in its ability to control infection. Other investigations, also aided by American Medical Association grants, seem to bear this out. Thus Smith and Sano (73*a*) reported in the Journal of Laboratory and Clinical Medicine in 1944, that water-soluble chlorophyll derivatives in concentrations of 0.05–0.5%, when added to tissue-cultures of embryonic fibroblasts, caused an almost immediate growth response with elimination of the usual six- to 18-hour lag period. Moreover, this growth-stimulating effect could be maintained by replenishing the chlorophyll every two days.

In more extensive and severe but also more indicative investigations, Smith and Livingston (73) in 1943 found that water-soluble chlorophyll derivatives accelerated the healing of experimental wounds and burns in animals. To obtain statistically significant findings, they studied the effect upon the healing of 1,372 experimentally induced wounds and burns produced by topical application of 17 medicinal preparations in current use. Of the agents tested, five were water-soluble chlorophyll derivatives in different vehicles. To further strengthen their findings, a control series of 872 similar lesions was also studied. It was found that of all the agents tested, only the chlorophyll preparations consistently showed any statistically significant effect on accelerating the healing of wounds and burns. Wounds thus treated healed 24.9% faster on an average than those treated with other preparations. The rate of healing was appreciably hastened in 67.9% of the animals on which the various chlorophyll preparations were used. This percentage, it was noted, was approximately twice that obtained with any of the other preparations studied and five times as great as that obtained with various sulfa compounds.

Combes (18) gives his results on the treatment of 103 patients afflicted by various dermatosis and cutaneous wounds, with water-soluble chlorophyll derivatives. To test the bacteriostatic efficacy, subjects with pyoderma, ecthyma and secondarily infected dermatosis were chosen. Patients with ulcerations, traumatic wounds of various types and compound fractures were also included in order to evaluate the wound-healing properties of the medication. A third group, including atopic dermatitis, dermatitis denenata, dermatitis herpetiformis, pemphigus and hypostatic dermatitis, was studied to determine whether any antipruritic and/or soothing effects would be produced and to see whether the application of chlorophyll hastened healing of the dermatosis as had been frequently demonstrated in ulcers. Many patients included in the study had received prior therapy with unsatisfactory

results. The undiluted chlorophyll solution was used as a wet dressing or soaked for half an hour at least four times a day. The ointment was applied daily or every second day in those cases where it was thought advisable to disturb the dressing as little as possible. Eighty-four patients were treated with the ointment alone, eight with the solution alone, and the balance with both preparations. In the last group therapy was initiated with wet compresses or soaks. The ointment was substituted when the lesions became dry. Combes concluded as follows:

a) On clinical trial chlorophyll solutions and ointments appeared to be effective agents in facilitating growth of granulation tissue and epithelization.

b) Both appeared to be effective bacteriostatic agents as judged by the Clinical Reduction Separation in the secondary affected ulcers, contact dermatitis, pemphigus, etc.

c) They had a moderate to marked anti-pruritic effect.

d) Under the conditions of these clinical trials both were non-toxic and could be classified as having a relatively low tendency to irritate.

Combes commented further that one outstanding benefit from the use of chlorophyll derivatives was its marked deodorizing effect.

Lowry (46), of the Department of Surgical Research, Ohio State University, reported the results of treating the following:

I. An unhealed burn of the leg of 15 years duration with an ulcer superimposed on the scar.

II. A breaking down of the skin of the leg in a case of elephantiasis.

III. An unsuccessful skin graft following a crushing hand injury.

His conclusion was as follows:

Three cases have been presented which demonstrate an effective healing response to chronic resistant skin lesions. The time involved in accomplished healing in one case does not detract from the fact that healing did occur in a lesion of 15 years duration in an ambulatory patient destined to work in an unfavorable environment. The successful healing of the ulcer cannot be attributed to chlorophyll alone. While it played an important part, it is felt that the infiltrations of bacitracin solution were a definite factor in eventually bringing about a state of repair. The case actually illustrates an effective cooperation between both agents. Case II emphasizes the epithelial stimulation properties of chlorophyll, and contrast of it against Case I illustrates the advantage of bed-rest and extremity elevation. Finally, Case III, while not a condition of long standing, is an instance of effective healing following an apparent failure to respond to skin grafting.

In 1938 Barnes (4), of the Hahnemann Medical College and Hospital, published a paper in which he compared 13 healing agents, among which were penicillin and sulfanilamide. Of all the agents tested, chlorophyll showed the greatest healing action and the most rapid healing rate. Barnes has continued his work, and several other reports by him demonstrate that measurement of the decline of electrical potential during the healing process serves as an index to the rate of wound healing. Thus Barnes was able to evaluate the effectiveness of chlorophyll and other healing agents by comparing the healing index of wounds treated with the test agent and the healing index of control wounds. By subtracting the percent lost per hour in the test wound, Barnes (4*a*) obtained the healing differential of the test agent. In one series of tests with experimental finger abrasions, water-soluble chlorophyll demonstrated a healing differential of plus 1.30, indicating a shorter healing time for chlorophyll-treated wounds than for the control. Chlorophyll's healing

differential was higher than that of any of the other agents tested which included shark liver oil, scarlet red, penicillin and sulfanilamide powder. In 1951 a further study by Barnes, reported in the American Journal of Surgery, reaffirmed the positive healing differential for chlorophyll against minor differentials for scarlet red, sulfanilamide and hydrosulphol. He commented: " Dressings containing chlorophyll were found to give a higher healing index (percent wound potential loss per hour) than dressings containing the same ointment vehicle alone when worn on the same person at the same time ".

Bowers (11), of the U. S. Army Medical Corps, reported in 1947 on the observations of 35 medical officers of the staff of the Winters General Hospital regarding the use of water-soluble chlorophyll ointments in over 400 clinical cases of wound healing. The doctors stated that granulation tissue was formed more rapidly and that it showed finer texture than with any other agent used. Epithelization also appeared to be stimulated more by chlorophyll than by other agents, including penicillin. In suppurating cases, including osteomyelitis and thoracic empyema noted for prolonged suppuration, chlorophyll caused cessation of pus formation in two or three days. In nasal and sinus cases chlorophyll had a quicker effect in stopping purulent drainage than any other agents. The author concludes: " Chlorophyll is the best agent now known for the use in the treatment of suppurative diseases, indolent ulcers or wherever stimulation of tissue repair is desired ".

These general clinical reports on chlorophyll in wound healing in humans confirm earlier work of the late Dr. Benjamin Gruskin (33) of Temple University, who in 1940 published (33) results on 1200 cases ranging from acute infections of the upper respiratory tract and sinus to chronic ulcerated lesions of various types. In his report Dr. Gruskin stated: " It becomes apparent that the use of chlorophyll in any acute or chronic process has resulted in definite improvement ".

In 1949 Carpenter (16) reported on the use of chlorophyll in the treatment of decubitus ulcers, particularly in paraplegics. He stated: " Cholorphyll derivative preparations demonstrated tissue stimulating properties, with subsequent early epithelization not previously seen in this particularly slow and indolent type of ulcer ". Carpenter also noted that " chlorophyll ointment used locally as an adjunct to the treatment of varicose ulcers, previously resistant to all forms of treatment, demonstrates remarkable therapeutic properties in every instance ".

In the British Journal Lancet, Bernard Niemiro (55) reported on the healing of pilonidal cysts, another link in the evidence of the ability of chlorophyll to accelerate the healing of wounds. As Dr. Niemiro points out: " The effectiveness of chlorophyll is clearest in calcitrant wounds which have a history of very slow healing ".

Ozena. Finocchi (26) reported that daily application of sodium chlorophyllin in isotonic salt solution to nasal ozena caused immediate improvement and eliminated the putrid odor of the disease in ten to thirty days in ten cases. The characteristic inflammation was reduced.

Hypertension. In 1932 Dr. Burgi (13) published his book entitled " Chlorophyll as a Pharmaceutical ", in which he related his treatment of 112 patients inflicted with hypertension associated with arteriosclerosis. Burgi included the actual clinical cases with descriptions of patients before and after treatment. He administered water-soluble chlorophyll tablets three times daily over periods of from two to six weeks. Burgi noted that in all ten cases a distinct drop in blood pressure occurred. In most cases the blood pressure returned to normal. Burgi refers to work published by Gsell,

Schroeder and Madison, and by others who also have found chlorophyll preparations of value in treating hypertension.

In 1931 Angelo (1) reported the results of his clinical studies on chlorophyll. Angelo experimented with 50 subjects broken up into three groups as follows: Group I—ten convalescents with normal blood pressure; Group II—17 patients suffering from high blood pressure, arteriosclerosis and severe nephrosclerosis, all of whom had been hospitalized because of disturbances caused by hypertension; Group III—23 patients suffering from high blood pressure, arteriosclerosis and nephrosclerosis, but in contrast to Group II these patients were living a normal manner of life in their own home. The experiments were conducted by using water-soluble chlorophyll which was administered in an aqueous solution in doses from 0.18 to 1.5 grams per day to the total exclusion of other pharmacological treatments. Angelo concluded: " By studying the action of chlorophyllin on the blood pressure of normal and hypertensive subjects, the following observations were made: 1. Chlorophyll is well tolerated and does not produce toxic symptoms even when administered in large doses and for several months. 2. It does not have any special effect upon the blood pressure of normal subjects. 3. In hypertensive patients it causes a steadily progressive fall in the arterial pressure and, the improved condition of the blood pressure is accompanied by a feeling of well-being ".

In 1932 Zickgraf (89) reported as follows: " Of the 18 cases treated by myself, only two cases of arteriosclerosis of the brain were not influenced in respect to blood pressure. As for the effect of chlorophyllin in the case of arteriosclerosis, many publications exist. Lowering of the blood pressure by widening of the arterial vessels, long lasting effect of chlorophyll, strengthening of the heart strength, besides the general tonic properties of the chlorophyllin, has been observed ".

Potential Therapeutic Uses. Chlorophyll compounds which belong to the chloro-porphyrins have a very amazing property which can be utilized for their future application in cancer therapy. When injected into test animals with induced and transplanted mammary carcinoma or sarcomas, the porphyrins concentrate in the tumors. These substances also concentrate in incised or otherwise traumatized parts of the body, accumulating in the regenerative margins of the incisions. This shows that growing tissues and regenerating parts in general have a definite affinity for the porphyrin-like compounds (25). This fact not only offers a key explanation for the healing effect of chlorophyll but has some significance for possible application of chlorophyll in tumor and cancer therapy.

The possibilities of cancer treatment with chlorophyll have already been investigated by Tixier (79), and also by Dupont and Duhamel (22) with encouraging results.

Considering the fact that chlorophyll derivatives can easily be labeled with, e.g., radioactive cobalt, and the ability of such strong chelates to accumulate in the neoplastic or malignant tissue gives this compound strong possibilities for use in detection and therapy of such tumors.

A further possible field of application for chlorophyll compounds as therapeutics stems from the ability of chlorophyll to chelate with a number of metals. Although this reaction takes place most effectively with bivalent metals, under certain processing conditions even chelates with trivalent metals can be obtained, if their coordination value is 2 or 4. This makes it possible to produce, aside from the already mentioned Co-chelates or Zn-chelates, also, e.g., Au-

chelates of chlorophyll. Such aurochelates would be very effective in gold therapy.

Gold therapy has been applied successfully not only in the therapy of tuberculosis but quite recently increasingly in the treatment of rheumatic arthritis (75). In the past this therapy has had the disadvantage of sometimes causing undesirable side effects. However, a chelating agent like the porphyrin ring releases its metal atom very slowly, and local over-concentration can thus be avoided. In recent years this type of therapy has become increasingly common (83); it has lately been extended to the therapy of lupus sepsis and syphilis. Because of the strong bacteriostatic activity experienced it is applied also to streptococcal or pneumococcal infections.

As a further development, the attempt could be made to synthesize halogenated chlorophyll chelates, e.g., chlorinated or iodized Cu- and/or Co-chlorophyllins. The therapeutic value of such organic iodine products was recognized recently in the treatment of rheumatic arthritis.

Analogous iodine-chlorophyllins as Cu- or Bi- chelates in treatment of heart disease and cancer treatment have been claimed by Melzer in a German patent (51).

Deodorant. One of the most outstanding contributions to clinical practice has been chlorophyll's remarkable efficiency in deodorizing foul smelling lesions, such as chronic osteomyelitis, chronic leg ulcers, infected compound fractures, deep sinus tracts, decubitus ulcers and lung abscesses. It is believed that cholorphyll's deodorizing action in the depth of a wound where odor-producing anaerobes may thrive is analogous to its action in plant metabolism. In a wound where suppuration is present, chlorophyll may, through the break-down of carbon dioxide and liberation of oxygen, deprive the anaerobes of the environment required for their survival. In this connection, a few years ago, there appeared in scientific journals several papers indicating chlorophyll to possess remarkable deodorizing properties. These papers contain evidence to substantiate the claim that chlorophyllin, when taken internally and in adequate quantity, reduces or eliminates offensive body and breath odors. Immediately after the appearance of these contributions commercial chlorophyll deodorant products were presented to the public. These initial products were effective as internal deodorants but only when taken in adequate quantity. Soon after the appearance of these first products, chlorophyll was seized upon by the promotion and advertising men who, in most instances, have completely distorted the facts of chlorophyll and incorporated it into every type of product ranging from toilet paper to hair restorers and fertilizers, with claims for such products backed only by the combined dreams of these same men. Because of these unfounded, fantastic and sometimes completely idiotic claims made for chlorophyll, some of the buying public has become skeptical of chlorophyll in all capacities, and they are eyeing all green products with an equally bilious eye. Because of this skepticism it is essential that the facts of chlorophyll as a deodorant be reviewed.

In March, 1950, Dr. F. Howard Westcott (85) reported: " In 1941, our studies were started with various fractions of water soluble chlorophyll to find an adjunct in the treatment of hyperchromic secondary anemias. Experiments were carried out on white mice and guinea pigs for one year. After proving conclusively that our fractions were non-toxic as well as hematopoietically effective, we studied various types of secondary nutritional anemias in humans. During the course of our studies in human anemias we had occasion to note that the odors

of foods and Vitamin B_1, usually detectable in urine, were greatly decreased when patients were taking our specially prepared chlorophyllins. This definitely indicated that some change in the metabolism of these odorous compounds was taking place and led us to study its effect on perspiration and body odors. Preliminary studies indicated that the water soluble chlorophyllins effectively reduced or eliminated the odor of benzyl mercaptan, thioglycolic acid and trimethylamine. Preliminary 'in vivo' tests were run on persons with nursing or medical backgrounds who could properly evaluate the clinical effects of this substance on body and breath odors. A physician and four nurses were trained to use the osmoscope until their results were constant. Following this test period the same group was given increasing doses of specially prepared chlorophyllins and readings were taken 24 hours after a bath ".

The results of these tests showed that specially prepared chlorophyllins reduced detectable under-arm odors from 56 to 100% in all five patients within seven hours and were effective as long as 18 hours in doses from 65 to 200 mg. To confirm these tests, a group of 12 college girls, including four negroes and eight white girls, were given these same tests for several days in succession while running experiments on the effect of the chlorophyllins on breath odors. One tablet, containing 100 mg. of the extract, was taken in the morning before moisture had a chance to dry on the skin surfaces; the perspiration odor was not detectable in over 90% of the cases and barely detectable in one case. Dr. Westcott went on to say: " Since these controlled studies, over 50 more individuals have been given the tablets and requested to report their own objective observation on its effect. To date, there have been favorable reports from over 90% of the subjects from individual observations on the relief of body odors. The dose needed has been 100 mg. daily, usually taken at breakfast or immediately thereafter ". This shows that the specially prepared chlorophyllin tablets taken by mouth are effective in neutralizing the obnoxious odors of the body for perspiration, and the effect lasts 18 or more hours. To test the efficiency of the specially prepared chlorophyllins on known breath odors, 12 subjects were given varying doses of grated onion or onion juice by mouth. Tests were run at 30, 60 and 120 minutes to observe the speed of deodorization. Sixty-two to 200 mg. of the specially prepared chlorophyllin powder were allowed to dissolve in the mouth, followed by swallowing of the residue. The results are summarized as follows:

a) When onion is chewed, then swallowed, the specially prepared chlorophyllin tablets chewed 15 minutes later caused an immediate cleansing of the breath. The odor of onion returns as soon as residual particles of onion remaining in the teeth crevices are digested and release more volatile oils.

b) When onion juice was used and no particles remained in the teeth to produce later decomposition products, the odor was barely detectable by direct smelling after two hours in one case, absent in four and definitely present in seven cases.

c) A similar study was made except that a mouth wash of specially prepared chlorophyllins in solution was used immediately after taking onion juice; results were more satisfactory and showed an immediate decrease in odor with osmoscope readings from 256 to 27 after five minutes.

This study reveals that the odor of onion remaining after eating solid onion is due to particles remaining in the teeth and crevices of the mouth. Careful rinsing of the mouth with the specially pre-

pared chlorophyllin solution or dissolving a tablet in the mouth would make it more permanent, but it is effective only if the mouth is cleansed.

Summarizing Westcott's experiments with specially prepared cholorphyllins, the following claims can be substantiated:

a) Chlorophyll fractions used in these tests are entirely non-toxic and suitable for human ingestion.

b) In vitro experiments have shown their effectiveness in deodorizing malodorous substances of neutral, acid or base reactions.

c) In vivo, they effectively neutralize obnoxious odors in the mouth from foods, beverages, tobacco and metabolic changes.

d) They effectively neutralize the obnoxious odors of perspiration due to physical exercise, nervousness and illness, obnoxious foot odors, menstrual odors, many urine odors and that of ingested materials.

In November of 1950 a report by Royal M. Montgomery and Henry B. Nachtigall (53) indicated that water-soluble chlorophyllin, when given in dosages of 200 mg. daily, effectively reduced noticeable underarm odor in both industrial workers and in college athletes. They also found that the chlorophyllin in doses of 300 mg. daily reduced the odors from pathologic conditions. In their first experiment a group of 25 employees from a large New York industrial plant was selected to represent the usual activity of the working person. A second group of 20 college athletes from a large university was selected for the study of perspiration odors of persons after vigorous exercise. A third group of selected cases was chosen to demonstrate odors due to various illnesses. At noon of the first day of testing, a control reading of underarm odor was obtained by use of the osmoscope. The average reading for the 25 employees was 2.3. Two 100-mg. tablets of chlorophyllin were given to each individual, one to be taken before retiring, the other after breakfast the next day. At noon on the second day the average reading for the entire group was 0.5. This represented an approximate 30% drop in 24 hours in measurable underarm odor following ingestion of 200 mg. of chlorophyllin. The dose was doubled the next day, the two 100-mg. tablets to be taken at night and two after breakfast the next day. At noon of the third day the average reading for the entire group was 0.2. This represented 91% decrease in measurable underarm odor for an accumulated dose of 600 mg. of chlorophyllin in 48 hours. Following this noon reading the distribution of tablets was altered without the knowledge of the testing group. Eleven individuals were given six 100-mg. chlorophyllin tablets, while the remaining 14 employees were given six placebo tablets. The routine method of ingestion was unchanged, one tablet at night with one tablet after breakfast the next day. This was sufficient for three days. At noon of the sixth day readings were taken of the two groups. Group one of 11 subjects with an accumulated dose of 1,200 mg. chlorophyllin in 144 hours showed practically a total reduction of underarm odor too faint for actual measure. Group two of 14 subjects who started with a 48-hour administration of chlorophyllins, but then were placed on a three-day placebo replacement, showed an average reading of 1.8 or a 75% return of the original degree of measurable underarm odor. The 72-hour period of placebo intake was estimated as being sufficient for elimination of the previously ingested 600 mg. of chlorophyllins by members of this group.

A group of 20 college basket ball players were tested by two physicians using the Fair-Wells metal osmoscope. Control tests were done for the normal

readings at 2:00 P.M. after their usual classroom work, and a second reading immediately after at least one hour of strenuous basketball practice. All the subjects were perspiring profusely at the second reading. About 20 readings were made on each subject during the study. After taking their normal reading, which was used as the control, each athlete was instructed to shower, using plain white soap, and to wear clean underclothes for each two-day period. They were given clean gym suits at the beginning of each two-day test to avoid accumulated perspiration odors. For the first two days each student took a 100-mg. tablet of chlorophyllins at breakfast. At 2:00 P.M. on the second day the osmoscopic readings were made. Readings for these tests were obtained as previously described from the armpits. After one hour of strenuous exercise the readings were taken again. On the third and fourth days the dosage was increased to 200 mg. of chlorophyllins. At breakfast 100 mg. were taken and the other 100 mg. at noon. For the 96-hour period the accumulated dosage was 600 mgs. The dosage was reduced on the fifth and sixth days to 100 mg. taken at breakfast. The accumulated dose for the six-day period was 800 mg.

The average reading for the 20 athletes before ingestion of specially prepared chlorophyllins was 1.5. Following one hour of strenuous exercise this reading rose to 3. After 48 hours of medication the osmoscopic reading was 0.9, before exercise, a 40 percent decrease in measurable underarm odor. Following one hour of basketball practice the osmoscopic reading was 1.2, a decrease of 60 percent from the control reading of 3.

After 96 hours of medication with the accumulated dosage of 600 mg. of chlorophyllins the reading before exercise was 0.15 and that taken after exercise was 0.3. Both were reduced 90% as compared to the controls.

The readings taken 144 hours after the ingestion of a total of 800 mg. of chlorophyllins showed that the decrease in measurable underarm odor before exercise was 76% and after exercise was 83%. The osmoscope readings were 0.36 and 0.5, respectively.

To further prove the effectiveness of chlorophyllin, the basketball players were divided into three groups for a two-day test period. Group 1 was given 200 mg. daily of raw chlorophyll; Group 2 was given 200 mg. daily of a copper salt of chlorophyll; and Group 3 was given nothing. The group which received natural chlorophyll showed a greater increase of measurable underarm odor. The group given chlorophyllins showed a decreased underarm odor before and after vigorous basketball exercise. A reduction of chlorophyllins to 100 mg. daily on the 5th and 6th days instead of 200 mg. resulted in a slight increase of measurable body odor. In the third experiment a total of nine cases included a hemoplegic with a large varicose ulcer; an 83-year-old female with large decubitus ulcers; a Negro with chronic nephritis; a woman with a large necrotic radiation ulcer on her knee; a woman with gangrene of the toe; a man with chronic hepatitis; and a 74-year-old lady with hemiplegia and inoperable cancer. A physiotherapist who had a body odor following daily exercise was also included. The odors were all greatly decreased except in the case of the radiation ulcer.

In March of 1952, Dr. John A. Killian (42) reported to the Society of Cosmetic Chemists the results of five series of experiments designed for in-vitro testing of deodorants. He summarized as follows: "Results of these assays have demonstrated that natural chlorophyll and its water soluble derivatives, sodium magnesium and sodium copper chlorophyllin, exhibited maximum efficiencies as deodorants for test conditions in which the

odorous compounds in the vapor phase are exposed to either the natural chlorophyll or the combination of chlorophyllins *a* and *b* in solid phases. In order to eradicate odors arising from surfaces of soluble matter it is essential that the chlorophyll derivatives be either water soluble or dispersable in colloidal form in the liquid to which it is added. The deodorant capacities of water soluble chlorophylls *a* and *b* are dependent upon the base acid balances of the solutions of the odors. The chlorophyllins exhibit deodorant actions on the neutral or alkaline solutions. The range of base acid balances which are optimum for deodorant effects of the chlorophyllins is indicated by levels of pH between 8 and 10.5. Even weakly acid solutions inhibit the deodorizing actions of chlorophyll. Water soluble chlorophyllins *a* and *b* added to malodorous samples of perspiration effect prompt eradication of the perspiratory odors. At the same time, the chlorophyll derivatives exhibit antibacterial action. In one series of tests 0.100% of chlorophyllins added to the samples effected a reduction of more than 99% in the numbers of viable bacteria in stale perspiration. In addition to their deodorizing actions, the chlorophyllins, added to fresh samples of perspiration, inhibit the development of perspiratory odors under conditions of storage which are optimum for the production of obnoxious odors ".

The series of experiments which have been cited in the preceding paragraphs exemplify the type of problems to which in-vitro methods of testing may be adapted and concerning which the intelligent utilization of these procedures may yield basic information of practical value, particularly in formulation of deodorant preparations. However, limitations of in-vitro techniques necessitate the adoption of in-vivo methods for purposes of either evaluating the deodorant efficiency of the final product or in comparative studies of two or more preparations on the market.

Dr. Serling, of Serling Laboratories, Inc., Larchmont, New York, indicated in 1950 (71, 71*a*) that chlorophyllins when incorporated into dog feed successfully eliminated or reduced unpleasant breath and body odors in dogs. Dr. Serling ran tests on dogs with a variety of breath and body odors, using chlorophyllin tablets, and discovered that the odors were greatly reduced in a very short time. His early research was followed by further studies for the Quaker Oats Company which involved months of feeding tests on all types of dogs, breeds, ages and with a variety of offensive odors. In 1952 Dr. F. N. Peters (59), Director of Research for the Quaker Oats Company, was quoted as follows: " Dog food containing chlorophyllin works fastest on breath odor, which is usually improved in three to six hours. Then its effect on body odor begins. In most cases an improvement in body odor is shown in six hours, although this may require as high as 24 hours. Both breath and body odors continue to diminish with additional feedings until they finally reach an irreducible minimum. Another advantage is that the objectionable ' wet dog ' odor of a dog that has been in the rain, or been given a bath, is usually eliminated. So, too, usually is the odor of an unhealed wound ". Among other interesting cases reported by Dr. Peters was that of a dog with no noticeable body odor itself, but which left an objectionable odor after sleeping on furniture or bed. The deodorant food eliminated this completely.

In our own laboratories extensive tests involving many dogs have been conducted. Sodium magnesium chlorophyllin, sodium copper chlorophyllin, magnesium-free pheophytin, alkali hydrolyzed alfalfa meal and several chlorophyll-containing commercial products have been tested. It was found that 27

mg. of sodium magnesium or sodium copper chlorophyllin per dog a day resulted in either elimination or substantial reduction in their odor (body and breath). It was also noted that 100 mg. of sodium copper chlorophyllin given to each dog per day did not reduce the odor any faster nor to a lower level than 27 mg. of sodium copper chlorophyllin. A commercial product containing sodium magnesium chlorophyllin was also effective. Natural chlorophyll (oil-soluble) was not effective under our conditions. Hydrolyzed alfalfa meal, regardless of the quantity given, was ineffective. In all experiments where the product showed deodorant activity it was noticed that the effect became apparent the second day after treatment. In most instances there appeared to be a general reduction in odor for a period of five or six days. Thereafter the odor appeared to level off with no further reduction. We believe it was significant that after we had deodorized a group of dogs they were taken off the chlorophyll treatment. The control (receiving no chlorophyll) group used in the previous experiment was then given the chlorophyll. As would be expected, those dogs receiving no chlorophyll increased significantly in their odor, whereas the control dogs were reduced in their odor intensity. In all our experiments the odor intensity was investigated in duplicate; usually the second experiment was run from one to two weeks after the first experiment had been completed.

In December of 1952, Joseph W. E. Harrison (36), of the Wall and Harrison Research Laboratories, Philadelphia, and the Department of Pharmacology, Philadelphia College of Pharmacy and Science, reported the following:

"*a*) A practical method has been described to evaluate the effect of deodorizing agents on the breath, which assimilates field conditions with maximum possible standardization. The odor sensitivity and olfactory fatigability of the evaluating judges was constantly tested to maintain control. The reproducibility of results obtained with this method is illustrated in a series of experiments on the effect of the water-soluble chlorophyll preparation which has been a collective investigation conducted by our four research groups; all groups took part in every phase of the study.

b) Application of this method to products such as chewing gum or lozenges containing water-soluble chlorophyll has established the following: A. The use of flavored gum containing four mg of water-soluble chlorophyll effectively reduced breath odor following ingestion of onions or beer or from smoking cigarettes. B. The use of a flavored gum containing a water-soluble chlorophyll reduces breath odor more effectively than does the use of a flavored gum without chlorophyll. C. The use of an unflavored gum containing chlorophyll also reduced breath odors more effectively than does the use of an unflavored gum without chlorophyll. D. Lozenges containing the same quantity of water-soluble chlorophyll are likewise as effective. This group studied the effect of chlorophyll on onion, tobacco and beer breaths with satisfactory results in all instances".

The foregoing literature citations unquestionably show chlorophyll derivatives to be effective deodorants when taken internally in doses of 100 mg. or over per day. In our own laboratories we have analyzed many deodorant tablets, chewing gums and lozenges now being distributed. We have found that many of these chlorophyllin-containing products cannot be of value as a deodorant because of their low chlorophyllin content. We have found certain chewing gums to contain from only a trace to five mg. per stick. One well known cough drop contained only 0.242 mg. per loz-

enge. Another lozenge contained 1.38 mg. chlorophyllin, another as low as 0.1 mg. per lozenge.

Such concentrations of chlorophyllin in these products certainly have rendered them of little or no value as deodorants, and until chlorophyll-containing deodorant products are made that do contain enough active agent to substantiate the claims made for them, the buying public as well as the scientist will remain skeptical of their value.

Oral Hygiene. In 1940 Dr. Gruskin reported (33) that chlorophyll preparations helped to control successfully such mouth infections as Vincent's Angina and advanced pyorrhea. Following this lead, Goldberg tested water-soluble chlorophyll derivatives in solution and ointment form on a series of 300 cases of oral disease and reported his work in the American Journal of Surgery during 1943. On the basis of his findings he came to the conclusion that chlorophyll is almost a specific against the organisms responsible for Vincent's Stomatitis (trench mouth) because it regularly brought about complete recovery and much more promptly than any other agents. In pyorrhea the use of these products resulted in tightening of the teeth, cessation of bleeding of the gums and new tissue growth. Disorders due to anaerobic infection rapidly and invariably disappeared with a few applications of chlorophyll.

Perhaps the most recent study of chlorophyll in dental hygiene was made at Boys Town Clinic, Boys Town, Nebraska, and reported by McDonnall and Domalakes (49) in 1952. The study was conducted on 589 of the boys living in Boys Town. They were divided into three groups without selection, and according to ages only, so that a comparable distribution would result. One group was given an essentially neutral dentifrice containing water-soluble chlorophyllins, another was given an alkaline dentifrice containing water-soluble chlorophyllins, and the third group was given a control dentifrice, containing no chlorophyll. At the time of each examination the examiner did not know nor inquire as to the nature of the dentifrice used by each boy. The primary purpose of this investigation was to determine the influence of tooth brushing with dentifrices containing water-soluble chlorophyllins upon the course of "gingivitis". They concluded as follows: The effects of brushing with three different dentifrices upon gingivitis was studied in a group of 589 boys, ages 10 to 18 years, living in Boys Town. This group showed an unusually high degree of gingivitis before the study was initiated. The amount of gingivitis was greatly reduced after two months of regular and routine brushing twice each day. A further but lesser reduction in gingivitis was achieved after an additional seven months of brushing. The reduction in gingivitis occurred more rapidly in the two groups using the dentifrices containing chlorophyllin than in the group using the non-chlorophyll dentifrice. At the end of two months brushing, the reduction in gingivitis was 70% and 58% in the two former groups and only 28% in the latter. At the end of nine months the reduction in gingivitis was similar in all three groups, 82%, 77% and 68%. At the end of two months of brushing the scores of the boys using the chlorophyll dentifrices was significantly lower than the scores of those using the non-chlorophyll dentifrice. However, at the end of nine months these differences were reduced and were statistically not significant. It was concluded, therefore, that the reduction in gingivitis was significantly accelerated in the groups using the dentifrices containing chlorophyllin during the two-month period but that continued use resulted in no advantages over the non-chlorophyll dentifrice. These findings were substantiated by

analysis of the frequency distribution of the number of persons with no gingivitis, mild, moderate and severe gingivitis before and after brushing. The number of boys with no gingivitis and mild gingivitis increased while the number with high, moderate and severe gingivitis decreased more rapidly in the groups using the dentifrices containing chlorophyll than in the group using the control non-chlorophyll dentifrice. At the end of two months the difference was marked. After nine months the difference was negligible. The above findings were substantiated further by analysis of the number of boys who improved or became worse. At the end of two months a greater percentage had improved in the groups using the dentifrices containing chlorophyllin than in the group using the non-chlorophyllin dentifrice. After nine months of brushing, these differences were less prominent. Very few failed to show improvement in gingivitis after nine months of brushing. No significant differences in the effects of gingivitis were observed between the two types of chlorophyllin dentifrices used in the study.

Toxicity. By far the most common of the chlorophyll compounds employed in the treatment of internal lesions has been sodium copper chlorophyllin. This copper salt has the advantage of being stable, water-soluble and easy to manufacture. Chemically the compound belongs to the chlorin system.

The fact that copper is in general a cumulative toxic agent for the human body has aroused some thoughts of the harmfulness of its chlorophyll compounds if taken uncontrolled in large quantities. This recently was pointed out by Dr. Corwin of Johns Hopkins University.

Although the danger of copper poisoning is not so acute as suggested by Corwin especially in clinically controlled treatment, the fact that in a longer treatment some damage to liver function could occur, seems to suggest that other chlorophyll derivatives with a different metal atom as chelating agent would be more desirable.

That the chlorophyllins are of low toxicity has been amply demonstrated. For example, Edward Rentz (61) injected 0.001 to 0.4 gram per kilogram of body weight of sodium chlorophyllin subcutaneously into rabbits with no adverse effects; and Angelo (1) administered in aqueous solution doses from 0.8 to 1.5 grams of sodium chlorophyllin to human subjects per day with no adverse effects.

Dr. G. Zickgraf (89) reports that natural chlorophyll, due to its waxy nature, cannot be considered for parenteral application; only the water-soluble chlorophyllins can. The dosage for subcutaneous injection was determined after many experiments to be an optimum at 0.5 mg. single doses daily or three times weekly, according to the condition of the case. He states further: "For eight months I have treated a great number of sick people with chlorophyll injections and have obtained remarkable results".

Robert Dupont and Gaston Duhamel (22), in doses of one to 100 mg., injected chlorophyll derivatives intravenously or into the tumors of cancer patients with no adverse effects.

Bollman and Sheard (10) injected sodium chlorophyllin intravenously into dogs with no adverse effects.

In his "Chlorophyll as a Pharmaceutical" (13), Burgi mentions that chlorophyll administered orally in doses of one to two grams was without effect, and he states that he had never been able to produce an injurious effect upon rabbits in this manner. No toxic symptoms resulted from subcutaneous injection of 0.2 to one gram of sodium chlorophyllin per kilogram of body weight and the same applied to 0.1 and 0.4 given by intramuscular injection. However, death occurred from intravenous injection of

0.125, 0.13 and 0.15 gram of sodium chlorophyllin; 0.12 gram produced toxic results but the animal recovered; 0.1 gram was ordinarily borne without producing toxic symptoms. He states further: " Very likely, it would be possible also to discover toxic doses for subcutaneous and intramuscular application of very large doses; but the quantities given would have to be greatly increased. The same applies even more to administration by mouth ".

Buttitta (15) reported that subcutaneous injection of 2 mg. of sodium derivative of chlorophyll into normal persons slightly diminished bleeding time and the time required for the quick test, and markedly diminished the coagulation time of blood. This would indicate that 2 mg. at least can be tolerated by the human being.

In 1944 Smith (72) reported on the administration of massive doses of water-soluble chlorophyll to rabbits orally, intraperitoneally, subcutaneously and intravenously, with no toxic effect whatever. Regardless of the size of the dose, there was no elevation of temperature and no impairment of physical activity. There was complete absence of any toxic effect in guinea pigs, dogs, rats, cats and mice when these animals were given the same chlorophyllin solution intravenously.

In our own laboratories rats have been injected with large doses of the following compounds: sodium magnesium chlorophyllin, sodium copper, zinc, nickel and cobalt chlorophyllins, and, while the animals took on a green coloration which was apparent in almost every organ of the body, none of them died, and the green coloration disappeared on withdrawal of the treatment. It was significant that the chlorin-e metallo compounds imparted a green color to the animals, while the same metallo compounds of the less basic chlorins f and g did not color the animals.

Rothemunde (63) states that 300 milligrams per day of porphyrin has not been found to be toxic. In fact, using the tetra derivatives, he administered so much of it to rats that their ears turned green and yet no death occurred. But a massive dose injected in the dark, followed by exposure to light, proved fatal.

Smith (72) also reported that when 240 cc. of a 2% aqueous solution of chlorophyllin was given orally for three days to a normal, healthy, adult male there were no subjective or objective symptoms except for a slight increase in bowel evacuation, the stool being slightly softer than normal and somewhat discolored by the drug. In the same paper Smith reported on the treatment of two cases of sub-acute bacterial endocarditis with intravenous administration of 500 cc. of 0.5% chlorophyll solution daily for eight days with no resulting toxic symptoms or systemic reaction to the drug.

In the foregoing literature citations the failure to properly identify the specific chlorophyll derivative with which the experimental work was conducted, is indeed apparent. Such failure is undoubtedly partially responsible for the variations in the efficacy of chlorophyll as a therapeutic agent, as reported in the literature. Recent studies in both Japan (54) and Europe (84) indicate that the chlorophyll derivatives chlorin-e_4, chlorin-f, chlorin-g and the purpurins are the therapeutically active compounds. If such proves to be the case, the pros and cons for the efficacy of chlorophyll may be easily explained by the failure of the producers of chlorophyll to manufacture a commercial product consistent in its content of the therapeutically active chlorophyll derivatives.

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Utilization Abstract

Shuttles and Bobbins. In the art of weaving, the weft thread, that is, the thread which is woven between the lengthwise-running warp thread, is carried on a spool known as a "bobbin", and the bobbin is encased in a boat-shaped "shuttle". In high speed weaving the bobbin revolves at a high rate and the shuttle passes back and forth at considerable speed. When these two parts are made of wood, as they usually are, the wood must possess a combination of hardness, strength and toughness, and especially of the ability to remain smooth under constant friction. In Europe boxwood (*Buxus sempervirens*) has long served this purpose, and in the United States, two native woods supply the needs and have even been exported to Europe for such use in competition with boxwood. They are the two arborescent species of dogwood (*Cornus florida* in the East, *C. Nuttallii* on the Pacific Coast) and persimmon (*Diospyros virginiana*). In addition, hard maple (*Acer saccharum* and *A. nigrum*), Pacific madrone (*Arbutus menziesii*) and the five native species of birch (*Betula lutea*, *B. lenta*, *B. nigra*, *B. papyrifera*, *B. populifolia*) have been used for the same purpose, but not to so exclusive a degree as dogwood, 90% of the cut going into shuttles. (E. H. F.).

Caranday—*A Source of Palm Wax*[1]

Millions of these palm trees in the Gran Chaco of central South America, occupying parts of Argentina, Bolivia and Paraguay, offer an unexploited commercial source of a hard vegetable wax, potentially as great as that of carnaúba palm in northeastern Brazil.

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Introduction

There exists in Paraguay a species of waxy-leaf palm, commonly referred to as "caranday" (*Copernicia australis* Becc.), which in many respects is similar to the carnaúba palm (*Copernicia cerifera* Mart.) of Brazil. Both palms elaborate waxy coatings which reflect their respective environments. At first thought it would not seem that two such widely separated regions as the sertão plains of northeastern Brazil and the Gran Chaco would have much in common. To some extent this is true, but certain geographic and climatic similarities can be observed which are sufficient to account for the elaboration of the waxy coating in the palms that are indigenous to each region.

The carnaúba palm, which is the source of the carnaúba wax of commerce, is found principally in northeastern Brazil in the States of Ceará, Piauí, Rio Grande do Norte, and Maranhão. Although the palm is numbered in the millions and is found throughout an area of about one and one-half million square miles, the wax industry is centered in the States of Ceará and Piauí. The natural habitat of the palm is an area subject to torrential rains and floods during a part of the year, followed by scorching heat and fierce winds which dry and parch the soil to an extent that few plants are able to survive. The period of drought in Ceará may last for a year and sometimes longer. The carnaúba palm is able to exist in such an unfavorable environment by virtue of the fact that it possesses an extensive root system highly adapted to garnering during the dry season the little available water, and because of its ability to elaborate an extremely effective protective coating against excessive evaporation of water through its leaves. This protective coating is the source of the carnaúba wax of commerce.

It has heretofore been believed that only in northeastern Brazil could there be found an environment and a palm such as have just been described. However, portions of the Gran Chaco can be compared with the sertão plains of northeastern Brazil, and the millions of caranday palms in that area can be compared with the carnaúba palm. The primary difference is that normally the periods of drought are shorter in the Chaco than in northeastern Brazil, and until about a decade ago the caranday palm was not recognized as a commercial source of hard vegetable wax potentially as great as the carnaúba palm.

Like the sertão plains of northeastern Brazil, the Gran Chaco comprises a vast flat, semi-arid plain devoid of major topographic features. This vast plain extends from the west bank of the Río

[1] Reprinted from the Journal of the American Oil Chemists Society 30: 309–313. 1953, with extensive revisions by the author and the editor.

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FIG. 1. Typical caranday palms, *Copernicia australis* Becc.

Paraguay to the eastern foothill of the Andes and from the Río Salado in Argentina to the Santa Cruz plains in Bolivia, occupying parts of Argentina, Paraguay and Bolivia.

The Chaco is essentially a low-lying country of little elevation and less relief, and large areas are therefore inundated during the rainy season or are flooded by the rising Paraguay and its tributaries which back their waters into the lowlands. In contrast to the uniformity of

FIG. 2. Map of Paraguay, showing the portion of the country covered by the Chaco which extends into neighboring Argentina and Bolivia.

relief, the surface soils display considerable diversity, owing to varying distances from the rivers and the nature of the underground drainage. In general, the soils vary from fine sands to heavy clays which are often impregnated with salts during the periods of drought. North of the Río Pilcomayo and west of the Paraguay for a distance of 400 miles no stream has carved a channel in the recent alluvial sediments. Only sluggish meandering and ill-defined streams drain

the rainfall from this area. During the rainy season (November to April) the Chaco receives 20 to 55 inches of rainfall which floods vast areas and converts it into a lake-dotted land. This is followed by a season of desiccation (May to October) when little rain falls, lakes and water holes dry up or become saline, and the meandering streams become mere ribbons or dry beds.

The Gran Chaco lies approximately between 20° and 30° south latitude, and its climate is therefore subtropical to tropical with considerable variation in temperature. In the southern portion minimum temperatures are near freezing in winter, while in the northern portion frosts are practically unknown. Maximum summer temperatures are considerably in excess of 100° F. and may be as high as 110° in the northern portions. Sudden changes in temperature result from the rapid and rather frequent changes in winds from the hot north to the chilling south.

Owing to the variation in climate, soils and drainage, the natural vegetation of the Chaco is unusually non-uniform. At varying intervals are found scrubby and thorny bushland (monte), grasslands (campo), palm groves (palmars), hardwood dicotyledonous forests (monte duro) and swamps (esteros). The extent of each of these varies from small patches or islands to relatively vast areas.

The Paraguayan Chaco is a source of appreciable national wealth. Besides cattle and some agricultural products, the forests produce timber, tannin (quebracho) and essential oil (palo santo). The caranday palm is another important resource, which is but little exploited, principally for timber (telephone and telegraph poles, farm structures, etc.) and as a source of fiber (ropes, bags, hammocks, a wicker or rattan-type of furniture, etc.) and leaf straw for the manufacture of hats (5). Potentially the caranday is much more valuable as a source of hard vegetable wax than for any other of the aforementioned palm products.

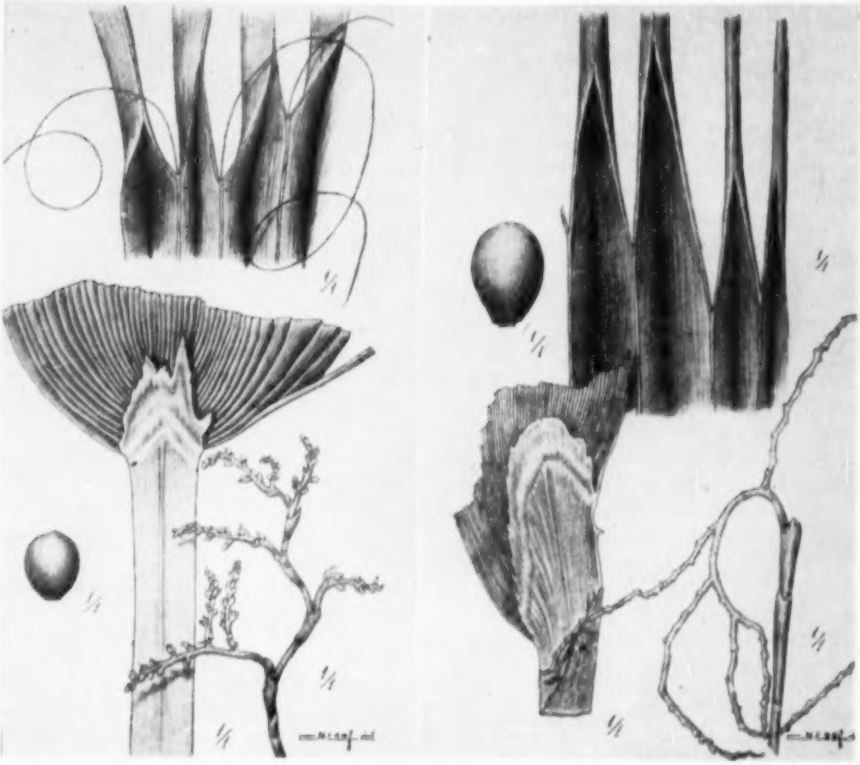
Although the caranday palm has long been exploited for a variety of purposes, its value as a source of hard vegetable wax has received consideration only in the past decade. This has probably been due to the isolation of part of the caranday area in Bolivia and Mato Grosso (Brazil), and in Paraguay to the greater economic importance of quebracho, palo santo and other trees in the accessible areas along the Río Paraguay and its tributaries.

In the course of a survey of the vegetable oil resources of Paraguay, the writer attempted to appraise the technical and economic possibilities of both small and large scale exploitation of the caranday palm as a source of hard vegetable wax. The pilot plants of two of the quebracho firms located on the Río Paraguay were inspected, laboratory records were examined, samples of wax were obtained and sent to various firms in the United States for analysis and evaluation, and several aerial and ground surveys were made of the distribution and extent of the caranday palm.

The Trees

Nomenclature and Characteristics. The caranday palm, also called "caranday", "carandá", "palma negra" and by other names, averages about 30 feet in height and has a trunk seven to ten inches in diameter. Although, in general, the caranday cannot be ranked with the tall palms of the world, occasional trees attain a height of 60 to 70 feet or more.* The young trees retain the spiny base of the leaves, but these are eventually shed and expose the dark and very hard trunk. Over wide areas the palm stems have a dark stain marking the level reached by flood water.

Superficially the caranday palm re-



FIGS. 3 & 4. Comparison of differentiating characters of the leaves, fruits and flowers of the caranday (right) and the carnaúba (left) palms, according to Kühlmann (5). About one-half natural size.

sembles the carnaúba of northeastern Brazil, and it is easy to confuse the two species. They have, however, been carefully described and distinguished by the Italian botanist, Beccari (1), who studied the genus *Copernicia*. Among the differentiating characteristics Beccari noted the following:

1. The leaves of adult plants in *C. australis* (caranday) have the surface covered on both sides with numerous minute reddish points or dots; the points are lacking or very few and scarcely visible in the adult leaves of *C. cerifera* (carnaúba).
2. In *C. australis* the branches of the third and fourth order arise inside a tubular funnel-form spathe; in *C. cerifera* there are only tubular spathes for the second order, and only from these arise the branches which then subdivide into floriferous branchlets.
3. The flowers in bud of *C. australis* are about twice as long as those of *C. cerifera;* in the latter the ovary is scarcely pilose at the top, it is densely pilose in *C. australis;* the style in the latter is slender and the stigma punctiform, while in *C. cerifera* the style is relatively thick

and the stigma distinctly 3-lobed; the stamens in *C. cerifera* form a fleshy ring with six very tiny teeth representing the filaments; in *C. australis*, on the contrary, the filaments have a broad triangular base which is abruptly contracted into a fairly long subulate filament; the tube of the corolla has six raised crests in *C. cerifera* and is smooth in *C. australis*.

4. The fruit of *C. australis* is smaller than that of *C. cerifera*.

Owing to the difficulty and confusion of distinguishing the two palms, Kuhlmann (2) prepared illustrations which permit a precise and easy means of recognizing and differentiating the two palms in the field or by means of herbarium specimens. These illustrations are reproduced in Figs. 3 and 4.

The first study of the anatomic structure of the leaf of the caranday palm was reported by Machado (4). The leaf material for this study was collected in 1943 in the vicinity of the Guaicurús Falls in Mato Grosso, but the results were not published until December, 1945, simultaneously with a study of the formation and properties of wax by de Medeiros Trancoso (3).

Occurrence and Natural Distribution. In order to determine the distribution and density of the caranday palm in the Chaco Borealis and to collect herbarium material, a total of 11 aerial reconnaissances were made between September, 1951, and November, 1953. The flights and ground surveys varied from one to four days and covered nearly all of the Chaco between 60° West Longitude and the Río Paraguay, and between the Río Pilcomayo and the Bolivian border; also along the western edge of Mato Grosso, Brazil, and the southeastern corner of Bolivia for a distance about 35 miles north of the boundary with Paraguay (see map).

Aerial photographs were made in many sections of the Paraguayan Chaco and on the ground wherever it was feasible to land. Herbarium or laboratory material was collected in many of the locations [3].

These surveys revealed that the caranday palm occurs over an area of several thousand square miles of the Paraguayan Chaco and extends north and northeastward into Bolivia and Brazil. In the lower Chaco along the Río Paraguay and its tributaries the caranday varies from thick stands, to highly scattered, to none at all. In general, it occurs in palmars (grass-palm plains), varying in extent from a few hundred acres to many square miles. Within some of the palmars may be seen forested areas (monte duro) which when viewed from the air appear like dark islands. Farther north the palmars give way to bushland (monte) which is characterized by a dense growth of thorny shrubs and cacti, interspersed with hardwoods and devoid of palms except in the vicinity of the rivers and other drainage channels. In the northeastern section of the Paraguayan Chaco above Bahia Negra, and extending into Bolivia and Brazil, the caranday occurs in solid stands hundreds of square miles in extent.

It was estimated that the largest palmar, which lies north and west of Bahia Negra and extending into Bolivia, contained possibly a half billion palms, and the entire caranday population was conservatively estimated at ten times as great as the carnaúba population of Brazil.

In general, the caranday palm is found throughout the Chaco wherever the land is subject to periodic flooding followed by complete drainage through run-off, seepage into the subsoil or evaporation,

[3] During 1953 a large amount of caranday material was collected, dried or preserved, and shipped to the United States for deposition with the Chicago Museum of Natural History where it forms a unique type collection.

and later to a protracted period of drying and parching of the surface layer of the soil. The palm is generally not found in areas above the flood level or in areas where submersion by water is more or less continuous. The periodic flooding to which the caranday areas are subject is probably the principal mechanism for distribution of the seed and hence spread of this palm. With the gradual raising of the alluvial plain through the erosion and redeposition of the eastern slopes of the Andes and the highlands to the north and east of the Chaco, the forest and bushland takes over and the caranday palm disappears. The transition of the flora with slightly increasing elevation is illustrated in Fig. 9. In the center of the photograph may be seen the water still remaining in the shrinking estero (swamp) as the dry season progresses. On either side may be seen the grass which apears as the estero recedes. Beyond and on either side of the treeless grassland are the caranday palms containing patches or islands of true forests (dark areas), which are above the flood level of the estero.

FIG. 5. Caranday palms invading and destroying grazing land through distribution of seed by cattle. Puerto Guaraní, Paraguay.

This ecological pattern is being disrupted in areas where cattle ranches have been established. In some of these areas the grazing land is being invaded by the caranday palm, Paraguayan mesquite (algaroba or inape, *Prosopis* sp.),

one or more species of acacia, and various mixtures of these plants. The palm is spread by cattle eating the fruit and subsequently regurgitating the seed during siesta or at night, often at considerable distance from the place of ingestion. This spread of the caranday palm and other exotic plants is further accelerated by frequent burning of the grasslands (Figs. 5 and 6).

The Wax

Pilot Plant Production. The earliest mention of commercial exploitation of caranday wax that has come to the author's attention refers to small-scale production in 1945 (3) by Ayrton Pacheo, employing primitive means at a point about 36 miles below Porto Murtinho on the Brazilian side of the Río Paraguay.

FIG. 6. Acacia and other exotic plants, in addition to caranday palm, invading grasslands through distribution of seed by cattle.

FIG. 7. Aerial view of a typical caranday palmar surrounding a darker hardwood forest. Each light-colored dot in the palmar represents a single palm tree, the trunks of some of which can be seen by means of magnification, and the dark dot accompanying each such light dot represents the shadow of the tree.

Subsequently the firm of Carlos Casado Ltda. became interested in the commercial exploitation of caranday palm as a source of wax. This firm operates a large quebracho extraction plant at Puerto Casado and owns about two and a half million acres of land in the Chaco along the Río Paraguay, which contains extensive stands of caranday palm. In 1947 the firm began an intensive laboratory investigation of the yield and variation of caranday wax in relation to the conditions of harvesting and drying the leaves. This investigation included the palms on the Chaco as well as the Brazilian side of the Río Paraguay in the vicinity of Puerto Casado. Subsequently (1949) a pilot plant for recovering wax was erected.

A second pilot plant was erected by the Sociedad Forestal de Puerto Guaraní, another quebracho extraction firm, located at Puerto Guaraní, 70 miles farther north. This installation included a building for drying leaves, two machines for separating the wax powder, a melting vessel and a centrifuge for separating the wax from the dirt and leaf trash. Sociedad Mercantil Colectiva " P.A.A.M ", S.A. Agro-Industrial y Comercial Espinosa Ltda. and other firms have developed equipment or experimented with the production of caranday wax.

Caranday wax has been produced on a pilot plant scale by two of the firms mentioned above. These plants have used various types of machinery for recovering the wax from the leaves after

drying them either in a special drying house or outdoors. The light gray, fluffy powder is removed from the leaves by aspiration with air while the latter are being cut and beaten in the machines. The machines used in the early operations had a capacity of 2,500 leaves per hour. The aspirated wax powder accumulates in a closed room or a bin into which the aspirator duct leads. In one of the pilot plants the powder is first purified, that is, separated from leaf trash and other suspended matter by bolting through fine cloth or woven wire screen before it is melted by indirect steam and filtered prior to running to small shallow pans where it is allowed to solidify. In another process the crude powder is melted with direct steam and hot water, and the extraneous dirt and leaf trash are separated by centrifuging. The separated liquid is run into large shallow pans where the water and wax separate into two layers as the wax slowly solidifies. After cooling, the cakes of hard wax, 0.75–1.5 inches thick, are broken into irregular pieces and bagged for shipment.

Yield per Leaf. Examination of the laboratory records covering harvests in 1947 and 1948 in the vicinity of Puerto Casado indicate that the yield of wax per leaf varied with the age of the leaves, weather preceding harvesting, conditions of drying, etc. Unselected leaves harvested after two or four days of rain and wind gave only 1.5–2.1 g. of wax [4]; selected leaves, i.e., no old or dying leaves, gave 3.8–4 g. of clean wax; unopened or olho leaves gave 3.5–4.4 g. of clean wax. Little difference was observed in the yield of wax from palms on the Chaco and on the Brazilian side of the Río Paraguay. Various authors have reported that the carnaúba palm yields 4 to 5 g. of wax per leaf, but none states whether the figures refer to crude wax powder or to clarified wax and, if the latter, of what degree of purity.

In comparing the relative yield of wax per leaf of caranday and carnaúba, the marked difference in the total surface areas of the leaves of the two palms should be borne in mind. According to Beccari, the mature carnaúba leaf is composed on the average of 60 leaflets, the central one of which is approximately 85 cm. in length and 35 mm. wide at a distance seven to eight cm. above the point of fission. The mature caranday leaf is composed on the average of 48 leaflets, the central one of which is approximately 65 cm. in length and 35–40 mm. wide slightly above the point of fission. At the same point the two outermost leaves are only seven to 15 mm. wide. If the data given by Beccari are representative for the two palms, it would appear that the total surface per leaf of carnaúba is appreciably larger than that of caranday. The leaf areas of carnaúba and caranday appear to be roughly in the ratio of 5 : 3, or, stated in another way, the total surface per leaf of the carnaúba palm is approximately two thirds greater than that of caranday, whereas the yield of wax is only one-eighth to one quarter larger.

During January, 1953, the author collected 250 leaves from palms found be-

[4] This product contained 3% total impurities but no water; all other samples mentioned were also free of water, contained 1% or less impurities, and melted at 84.5–85° C. Yields of crude wax powder were higher than the figures quoted for the clarified wax.

FIG. 8 (*Upper*). Aerial view in the Paraguayan Chaco of forest and bushland (dark areas) surrounded by caranday palms (lighter areas) along a meandering river.

FIG. 9 (*Lower*). Aerial view in the Chaco of changing flora with increasing elevation and drainage of the land. Center, more or less featureless, horizontal area indicates a receding swamp flanked by treeless grassland which is succeeded by palmar surrounding darker areas of hardwood. The light and dark dots in the palmar are individual trees and their shadows.

tween the Río Pilcomayo and Puerto Guaraní, and sent them to S. C. Johnson and Son, Racine, Wisconsin, for examination and analysis. Preliminary examination of all the leaves revealed that the olhos (unopened leaves) had a higher incidence of free-flaking wax than the palhas (opened leaves). Subsequent analysis showed that on an average the palhas yielded more total wax than the olhos. Individual leaves varied considerably in surface area and weight, as well as in amount of total and free-flaking or caducous wax.

On an air-dry basis, individual leaves had the following variation in weight: Lower Chaco, 60–121 g. (av. 82); Puerto Casado, 26–128 (av. 61); Puerto Guaraní, 67–88 (av. 72); Espinosa Estancia near Villa Hayes, 75–100 (av. 85). In order to intercompare the yields of wax it was necessary to express the analytical results on a basis of 100 grams of dried leaf material.

TABLE I

VARIATION IN TOTAL AND CADUCOUS WAX IN AIR-DRIED CARANDAY LEAVES

Nature of wax	Number of leaves analyzed	Amount of wax present	
		Range	Average
Total [a]	32	3.0 –10.6	6.8
Caducous [b]	15	0.4 – 5.3	2.7
Total, olhos [a]	17	3.0 – 7.95	5.8
Total, palhas [a]	30	5.28–10.6	8.5

[a] Determined by solvent extraction.
[b] Determined by mechanical beating.

The results of a limited number of determinations of total and caducous wax are given in Table I. Based on the examination of the 250 leaves, it was concluded that on the average, caranday leaves are much less free-flaking than are carnaúba leaves. Some caranday leaves had as much as four grams of free-flaking wax per 100 grams of dried leaf, but the average was only about one and a half grams.

Since these analyses were made, preliminary experimental work carried out at Estancia Deolinda by Nils Gustafson has demonstrated that rapid artificial drying results in a marked increase in the proportion of free-flaking to total wax compared to sun-dried leaves from the same palm. The increase in caducous wax appears to be related to the rate of drying (less than 24 hours compared to three to five days for sun drying), the final moisture content of the leaves, and the relative humidity of the surrounding air. A determination of the moisture content of an artificially dried and a sun-dried leaf from the same palm gave two and a half percent and twelve percent moisture, respectively.

Quality. When fractured, the hard wax exhibits a yellowish-green to light brownish-green color. It is readily bleached to pale tan by very small amounts of benzoyl peroxide and to pale cream by hydrogen peroxide-chromic-sulphuric acid.

Samples of the wax were submitted to various firms in the United States and abroad for examination and evaluation in product formulation tests. All but one firm reported that the wax was superior to ouricury and comparable to the better (T-3 to T-4) grades of carnaúba wax. The principal physical properties are shown in Table II together with similar data from the literature for carnaúba, ouricury and candelilla waxes.

On the basis of the usual laboratory tests it is apparent that it would be difficult to distinguish caranday from carnaúba wax. One firm reported that " in certain of the practical tests for finished polish product applications it shows up as well as carnaúba wax but in others it is somewhat inferior. In all of the polish product tests which were run, however, it was considered superior to ouricury wax ".

From tests which have been made by various end users there seems to be little

TABLE II

COMPARISON OF CARANDAY WITH OTHER HARD VEGETABLE WAXES

Characteristic	Carnaúba	Ouricury	Caranday [a]	Candelilla
Specific gravity 25°/4°	0.990–0.999	1.0661	0.990 [b]	0.982–0.993
Acid value	4.0–9.7	21–24	9.5	12–20
Iodine value	7–14	6.9–7.1	8.0 [b]	15–37
Acetyl value	54.8–55.2	.....	40 [b]	.....
Saponification value	79–95	61.8–85.3	64.5	46–65
Melting point, °C	83–85	79–84	84.5 [c]	64–71
Moisture, %	.....	.....	0.9	.....
Ash, %	.....	0.55–1.9	0.18	.....
Acetone-soluble at 25° C	2.0–2.5	.....	2.7	20

[a] Wax produced by Carlos Casado, Ltda. Analyses supplied by courtesy of S. C. Johnson & Son, Inc., except as otherwise indicated.
[b] Determined at the Southern Regional Research Laboratory, U. S. Department of Agriculture, New Orleans, La.
[c] Drop point method. Cryoscopic melting point 79.7° C.

doubt that the wax of caranday palms is as suitable as carnaúba for many applications, particularly in aqueous wax emulsion products.

Imports and Present Status of the Industry in Paraguay

In 1950, 1,297 kilos of caranday wax were imported into the United States under the classification "Paraguayan carnaúba". The wax was valued at $970 f.o.b. New York, or 75 cents per kilo, whereas the total import of carnaúba for that year averaged somewhat more than $1.70 per kilo (10). The low price received for caranday wax compared with carnaúba was one of several factors which resulted in closing the pilot plants and stopping further efforts to develop a caranday wax industry in Paraguay.

Another problem which confronted the producers resulted from the grant of a series of monopolistic patents (6–8) late in 1949 and early in 1950. The validity of these patents was questioned almost immediately through the medium of a suit brought by a potential producer of caranday wax. The suit was finally decided in favor of the patentee, but the patents were subsequently voided by a decree law (9).

Prospects for Profitable Exploitation

The results of the survey and investigations reported in the foregoing account together with other accumulated information suggest that profitable exploitation of caranday palm as an industrial source of wax should be technically feasible. Many of the larger stands of this palm are adjacent to the Río Paraguay, on which regular boat service is maintained and along which are situated towns and industrial plants. Roads, trails and narrow gauge railroads extend from the river into the Chaco at various points, thus making it possible to reach vast numbers of palms without difficulty. Furthermore, the relatively small height and high density of caranday palms, as compared with carnaúba, should make collection of their leaves fairly easy.

The problem of labor supply, especially for harvesting the leaves, is probably the greatest handicap to be overcome in developing a caranday wax industry. Such skilled workers, administrators and technically trained individuals as are located in the towns along the Río Paraguay are generally employed by the quebracho-processing plants. This is generally true of a large number of Indians who live in the bush and who are engaged in cutting quebracho. Cutting and

processing quebracho for tannin are year-round operations and are probably considerably more profitable than would be production of caranday wax; consequently it is unlikely that labor would be diverted from this source for cutting and processing caranday leaves. There are also many Indians employed on the ranches who may or may not be available at time for harvesting palm leaves. It may be possible to induce additional Indians from the interior to migrate, at least for the leaf-cutting season, to the vicinity of the palm areas. Since many Indians have been trained to be excellent hacheros (axe-men), it should be possible to train others to be equally good palm-leaf cortadores.

An item of very considerable importance to the establishment of a new industry is the attitude of the government with respect to taxation, export duties, rates of exchange, and the amount and degree of regulation which may be imposed on it, especially during its development stages. Given favorable governmental consideration and an adequate supply of efficient labor, the exploitation of caranday palm in the Chaco of Argentina, Bolivia and Paraguay could in time equal or surpass that of carnaúba in Brazil as a commercial source of industrially needed wax. Such a new source of hard vegetable wax would for many years remove the threat of a shortage as now exists and will continue to exist so long as carnaúba remains almost exclusively the backbone of the hard vegetable wax-utilizing industries.

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Utilization Abstract

Lavender. Lavender oil is commercially obtained from both wild and cultivated plants, chiefly the former, in southern France. The greatest use of this essential oil is in the soap industry; secondarily in the perfumery trade for manufacture of cologne waters and the preparation of a few perfume bases; thirdly, for medical and veterinary preparations. Also, dry lavender blossoms are the basis of scent cushions.

Wild plants grow at elevations between 800 and 6,500 feet in about 20 Departments of France, but mostly in that of the Drome (which has the highest production), of the High and Low Alps, the Maritime Alps, the Vaucluse and the Var.

Two forms of lavender (*Lavandula officinalis* Chaix) are recognized—*delphinensis* and *fragrans*—differing in flowers, foliage, growing preferences and nature of their oils.

Collecting the flowers is done by hand; 100 to 130 pounds per man per day from wild bushes, up to 900 pounds from cultivated plantations. The oil is extracted from the flowers by steam distillation and by solvent extraction. (S. J. Hugues, *Am. Perf. & Ess. Oil Rev.* *62(6): 433. 1953*).

Forest-tree Genetics Research: *Quercus* L.

Oaks are the sources, not only of the most valuable timber among broad-leaved trees of the northern hemisphere, but also of cork, tannin, a dye, and food for man and beast. Their prolonged pre-flowering periods and other factors have discouraged genetic investigation of them, but what little is known in that direction is here summarized.

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Introduction

From a genetical point of view, the genus *Quercus* L. has received little attention as compared with *Pinus* and *Populus*. One explanation obviously involves the long span of time required for oaks to reach flowering age; but there are other reasons as well. Controlled hybridization is not easily accomplished. Vegetative propagation is, if not difficult, at least time-consuming, and seed crops occur at irregular intervals. However, since oaks are among the most important hardwood timber crop trees in the northern temperate zone, some genetic research has been concerned with them, both in the New and the Old World. The results of these studies have been published in many widely scattered journals and bulletins, some with a very limited circulation. The present review is an effort to assemble some of the available data together with a few unpublished data from recent experiments. No claim for completeness is made.

The growth habit of *Quercus* varies from shrubs a few feet in height, dense and straggling in arid or alpine regions, to some of the largest woody plants in the northern hemisphere. For example, *Q. borealis maxima*, *Q. macrocarpa*, *Q. robur* and *Q. velutina* sometimes attain heights of 50–55 meters (**84**). The genus *Quercus* probably reaches its best development in the eastern United States. Immense semi-arid areas in northeastern Asia are also covered with oak forests of *Q. dentata* Thunb., *Q. mongolica* Turcz. and others (**106**), but they are often of rather poor form. All oaks are wind-pollinated and essentially monoecious. They hybridize more or less readily within the sections. Evergreen, semi-evergreen and deciduous species occur. Intensive silviculture of oaks has not been practiced in North America to any degree, due to the extensive resources. But in Europe oaks have been planted and cultivated intensively for centuries. There is no doubt that in the future, when our reserves of hardwood timber are reduced and the demand increased, general forest-tree improvement will be a necessity. North American forestry is in an intermediate stage between simple exploitation of forest reserves and intensive forestry. For the time being it may not look economically feasible to undertake intensive planting and silviculture, though opinions on this matter are rather divergent. Sooner or later we will be forced to enter upon very intensive forestry. Until then we will certainly do well to get acquainted with the genetics and silvics of our forest trees. The combined efforts of these two disciplines will enable us to make considerable progress

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in forest-tree improvement, an improvement which in time will be an economic necessity.

Classification and Nomenclature

Quercus is represented by between 200 and 300 species in the temperate regions of the northern hemisphere and in the tropics at high altitudes south to Columbia in America and to Malayan Archipelago in Asia (84). Classification within the genus presents many difficulties. Even the concept of the genus seems to cause controversy among taxonomists. Two of the most recent classifications are presented below.

A. Camus (14)

Quercus L.
- Subgenus Cyclobalanopsis Schneid.
- Subgenus Euquercus Hickel et Camus
  - Section Cerris Spach
  - Section Mesobalanus A. Camus
  - Section Lepidobalanus Endl.
  - Section Macrobalanus Oerst.
  - Section Protobalanus Trel.
  - Section Erythrobalanus Spach.

A. Rehder (84)

Quercus L.
- Subgenus Cyclobalanopsis Prantl.
- Subgenus Erythrobalanus Spach
- Subgenus Lepidobalanus Endl.
  - Section Cerris Loud.
  - Section Suber Reichenb.
  - Section Ilex Loud.
  - Section Gallifera Spach
  - Section Robur Reichenb.
  - Section Prinus Loud.

In 1936 O. Schwartz (92) raised certain of the subgenera to generic rank. It is beyond the scope of this article to discuss these taxonomic problems. In the following Rehder's classification will be used. The subgenus *Cyclobalanopsis* Prantl. occurs in eastern Asia and the Malayan Archipelago. Subgenus *Erythrobalanus* Spach, the so-called red or black oaks, is confined to North and Central America. Subgenus *Lepidobalanus* Endl., the white oak group, occurs in Asia, Europe, and North and Central America.

Also the species concept presents problems because of the great morphological variation in *Quercus*. Numerous papers dealing with variation in oaks have been published, e.g., Domin (22), Fiori (28), Hauch (37), Krason (48), Kurata (49), Landolt (50), Muller (61, 62, 63), Oersted (71, 72), Palmer (75), Petersen (76), Stebbins (94), Sylven (96) and Weimarck (107). Some species in *Quercus* seem not closely related to any other species, while still others are barely distinct from their closest relatives. If Rollins' "species standard method" (85) be applied to *Quercus*, a different "species standard" would have to be used even within the different groups. As an example of the extreme variation in *Quercus*, Lasch (52), as early as 1857, described no less than 67 forms of *Q. robur* and *Q. petraea*, including presumed hybrids. Vicisio (104) enumerates 23 forms of *Q. ilex* alone in Spain. Camus lists 44 forms of *Q. lanuginosa* Lamk. and about 50 forms of *Q. robur*. The above mentioned species have, of course, been subjected to detailed taxonomic investigations for decades; but the same amount of variation could doubtless be found in many other species. The creation of varietal names for all these forms introduces needless confusion, since the polymorphism doubtless indicates various phenotypic expressions of the same or very similar genotypes when grown in different environments. Muller (62) states that many of the Mexican species and varieties may prove to have been described from plants originating as stump sprouts, since such sprouts often show considerable variation in leaf shape. Stump sprouting is very common

in Mexico due to the primitive method of clearing land for agriculture. Many of the species occurring in arid regions propagate by sprouting from subterranean rhizomes. A single plant may form thickets up to 15 meters in diameter, as has been reported for *Q. havardi* Rydb. (65). Such clones are probably very old and serve to maintain the distinctness of the species, but may also preserve genetic differences which have arisen, as noted by Muller (65). He also mentions the so-called "mottes" in *Q. virginiana,* that is, clumps of even-aged trees, often of considerable size, originating from juvenile rhizomes.

Another factor contributing to the taxonomic confusion may be the practice of naming hybrids. According to the International Rules of Botanical Nomenclature, such a practice is allowed provided the possible range of variation in F_1's is indicated. F_1 hybrids between the same two species may, of course, vary considerably. This variation may even reach the point where "varieties" of hybrids are described (89). If this practice were followed, we would soon have thousands of varieties. When and if hybrid oaks are propagated vegetatively, the practice of using clonal names or numbers, as in the case of *Populus* hybrid clones, should be adopted. This suggestion may seem premature with regard to oaks; but in time we may be working with several hybrids or selected trees and thus avoid posible future confusion.

Utilization

The oaks represent the most important source of timber among the broadleaves species of the northern hemisphere (34). Oak wood for sturdy construction has been held in high esteem for centuries. The vikings of Scandinavia, some 1000 years ago, built their ocean-going boats of oak timber, some of which are still found well preserved in bogs of Scandinavia. That oak (mainly *Q. robur*) was for centuries considered a valuable timber tree in Europe is indicated by the role it has played in the folklore of many countries.

In 1938 the standing sawtimber supply of oaks in the United States was estimated to be 83.7 billion board feet, practically all in the eastern part of the country (103). This figure represents about 5% of the total standing sawtimber (1,601 billion board feet) at that time.

Other important oak products include cork from *Q. suber* L. and *Q. suber* var. *occidentalis* Arcang., both of which are indigenous to the Mediterranean region and produce about 282,000 short tons of cork per year (86). Attempts to introduce this species into the United States started about 1855 in the Southwest. From 1940–46 about 200,000 seedlings were distributed for planting in California, and in 1945, 16,000 pound of acorns from this species were collected in the United States. Although they show a germination of about 90%, natural regeneration is very poor (58). Tannin was in early days extracted from the rootbark of some European species. Later, extracts from the bark of younger trees were made. In North America the bark of *Q. borealis, Q. velutina* and *Q. prinus* were frequently used. Since the discovery of the quebracho tree (*Schinopsis* species) in South America, the wood of which contains much tannin, the use of oak bark tannin has decreased. In the Eastern Mediterranean region the two species *Q. macrolepis* Kotsch. and *Q. valonea* Kotsch. bear fruits with a very high percentage of tannin (the scales of the cup, ca. 45%; the cup, ca. 25%; and the acorn, ca. 16%). The fruits are sold under the trade name "Valonea". Galls caused by the wasp *Cynips gallae tinctoria* on *Q. infectoria* Olivier in Asia Minor frequently attain diameters of one inch and contain 70% tannin, which is used chiefly in the manufacture of ink

and some medicines. The wasp *Cynips insana* causes galls on *Q. robur* and *Q. lusitanica* weighing up to five grams and containing ca. 25% tannin. Pulverized they are sold under the name "Rowe". A yellow dye, quercitrin, is extracted from the yellow inner bark of the North American black oak, *Q. velutina* Lam. (33). In addition to these products, the scrubby oak species in arid regions play an important role in erosion control and constitute a major part of the chaparral of the Southwest. The fruits have been used as a source of food by the Indians in the Southwest. According to Schenck (90), those of *Q. haas* Kotsch. are very sweet and edible, and have been planted for this reason in Asia Minor. Attempts to select forms of *Q. robur* with edible fruits have been made in Europe, so far without success. The fruits of beech and oak have, for centuries, played an important role as food for hogs.

Natural Hybridization and Speciation

As previously mentioned, natural hybridization within the sections is known to occur. Numerous papers dealing with presumed natural hybrids have been published, e.g., Allard (2), Britton (10), Bush (12, 13), Camus (15), Davis (18, 19, 20), Duncan (24), Hollick (38), Kase (44), Kline (45), McMinn, Righter and Babcock (55), Palmer (74), Rehder (83), Sauer (87), Sudworth (95), Trelease (102) and Wolf (109). However, the frequency of hybridization has probably been overestimated, since large hybrid swarms are seldom found. Probably one of the first natural hybrids to be discovered was the "famous" Bartram Oak, described by F. A. Michaux in 1812 as a species under the name *Q. heterophylla*. Identity of this oak caused much debate among taxonomists for over 100 years, but it is now generally regarded as a hybrid between *Q. phellos* and *Q. borealis*. In the following years many other presumed hybrids were discovered and described. Thus Palmer (74) in 1948 enumerated about 70. This high number should not, however, be interpreted as an indication that hybrid oaks occur very commonly. For, even though hybrids or presumed hybrids can be found easily by careful searching, one will, as Palmer (74) puts it, "probably encounter hundreds of thousands or even millions of trees of the different species for every hybrid". Hybridity apparently seldom affects the distinctness of the species, in spite of the fact that many hybrids are fertile and a certain amount of backcrossing and segregation occurs. The word "apparently" is used as an indication of our inability to judge the importance of hybridization in speciation when the long span of time required for each generation is considered. Stebbins, Matzke and Epling (93), in an account of introgression of *Q. ilicifolia* into *Q. marylandica* studied in New Jersey, point this out by comparing *Quercus* with *Drosophila*. Under the assumption that the above mentioned two oak species have existed in New Jersey for the last 25,000 years, they assume that there have been only about 250 generations. If *Quercus* and *Drosophila* have the same rate of change per generation, then 25,000 years in *Quercus* would compare to about ten years in *Drosophila*.

Quite apart from the long generations in *Quercus* is the fact that fertile hybrids do not become readily established. One of the reasons may be traceable to the absence of suitable habitats. Muller (66) gives examples of such a situation in the case of hybrids between *Q. havardi* and *Q. mohrianea* Buckl. in Texas. The former species is strictly confined to deep sand, the latter to limestone outcrops. The hybrids occur only on soil composed of a mixture of sand and limestone fragments (on bluffs), although very slight introgression may be found at some distance from the zone of range

overlap. Other examples of the influence of edaphic factors on introgression are given by Muller in crosses involving *Q. mohriana* and *Q. grisea* Liebm., *Q. mohriana* and *Q. stellata*, *Q. incana* Bart. and *Q. marylandica*, *Q. margarettae* Ashe and *Q. stellata*. An example of climatic restrictions on gene flow is also given by Muller in the case of *Q. havardi* and *Q. stellata*. The former species is able to thrive on rather dry, sandy soil, whereas the latter is found in regions of higher precipitation in Texas. Where the ranges of the two species overlap (e.g., in Wheeler County, Texas), some gene exchange occurs, but, since the precipitation decreases westward, "*stellata* genes" are not found very far west of the zone of range overlap. If the environment were "hybridized" in the sense of Anderson (4) we would without doubt find many more hybrids. Hybrid oaks do, in fact, often occur where the habitat has been disturbed.

One of the commonest sequels to hybridization in many genera is repeated backcrossing of the hybrids to one or both parents. Hybridity therefore becomes less apparent, and the end result is increased variability in the two parent species. Without backcrosses survival in F_2 generations may be expected to be low, since only the plants most similar to the parents would be capable of survival in the old habitat.

The work of Epling (26) on gene flow in populations of *Arctostaphylos*, *Salvia* and *Delphinium* indicates that sympatric species in these genera are capable of maintaining their identity, even though they are sufficiently compatible to form natural hybrids and backcrosses. Stebbins (94) gives examples of hybridity in overlapping ranges of morphologically dissimilar allopatric *Quercus* species, while sympatric similar species show little or no indication of hybridization. He mentions *Q. garryana* Hook. and *Q. douglasi* Hook. et Arn. where hybridization occurs in the zone of range overlap. At the southern end of the range of *Q. douglasi* hybridization also occurs with *Q. dumosa* Nutt. and its variety *turbinella* Jepson so that there is evidence of gene interchange between a chain of forms ranging from a large tree with broad, deciduous, lobed leaves to a shrub with very small, coriaceous, evergreen, unlobed leaves. *Q. lobata* Nee, however, which is the counterpart of *Q. garryana* both in habitat and character of the tree, shows relatively little evidence of hybridization in localities occupied by these two species. Furthermore *Q. lobata* and *Q. douglasi* show very little evidence of intergradation with each other. Stebbins concludes that the magnitude of the visible differences between Californian oak species bear no direct relationship to the amount of intergradation that can occur between them.

Other and perhaps even more striking examples of gene exchange between morphologically dissimilar *Quercus* species are the hybrids between the evergreen, narrow-leaved and small-fruited *Q. virginiana* and the deciduous, broad-leaved and large-fruited *Q. lyrata*. According to Palmer (personal comm.), this hybrid occurs rather frequently in nature.

Proof of hybridity cannot be obtained with absolute certainty by comparison of morphological characters. A more certain way of testing hybridity is by a progeny test, as done by Allard (1, 3) with the naturally occurring hybrid *Q. alba* × *Q. montana*, by Bartlett (6) with ×*Q. deamii*, and by McDougall (54) who, in 1907, progeny-tested the Bartram Oak and came to the conclusion that it actually is a hybrid between *Q. phellos* and *Q. borealis*. In this connection must be mentioned the strictly sharp segregation into the parental types that may occur in the F_2 generations. Yarnell (112) explains this as partly due to linkage, especially because it is likely that there is less crossing over in the F_1

than in either of the parental species. Stebbins (94), however, by comparing this segregation in oaks with the segregation in other plant groups, is of the opinion that the number of genes by which species of oaks differ from each other is considerably smaller than in most other plant groups. Another method to test the hybridity of a presumed natural hybrid is, of course, to repeat the cross artificially. But even this may not be a certain method, unless the same parental trees are used.

There is no doubt that some claims for hybridity in nature may, upon detailed investigation, prove to be variations caused by stump sprouting, modifications due to ecological stress, or heterozygosity resulting from ancient crosses (62, 74).

Selection from Wild Stock

One of the simplest and most obvious methods of tree improvement is, of course, selection from wild stock. Apparently such selection has been undertaken only on a very small scale in the United States or Canada, if at all. In Europe, on the other hand, one of the first authentic records on selection in oaks appears in Johan Colerus' "Oeconoma rivalis", published in 1606. In 1662 John Evelyn wrote in his "Sylva or Discourse of Forest-Trees, and the Propagation of Timber in His Majesties Dominations" (London 1670, pp. 8 and 20): "chuse not your seed alwaies from the most Fruitful-trees, which are commonly the most aged, and decayed; but from such as are found most solid and fair: Nor for this reason, covet the largest Acorns, etc . . . (but as Husband men do their Wheat) the most weighty, clean and bright". Discussing the different forms of trees, he continues: "the difference proceeding doubtlesse from the variety of the Seed, and therefore great care should be had of its goodnes, and that it be gather'd from the best sort of Trees, as was formerly hinted".

Three to four hundred years ago oak timber played a very important role in the ship-building industry, especially the building of war ships. For this reason the monarchs of many European countries were interested in the supply of this valuable tree, and various laws were enacted for the sole purpose of acquiring and protecting oak forests.

Selection has been practiced in Europe for many years through collection of seed from phenotypically superior trees, or, more often, by mass collection from areas with superior oak forests. Such activity has led to the consideration of provenance. Oppermann (73) mentions that extensive plantings of oak in Denmark started in 1700–1780. Because of lack of the necessary seed material, acorns were imported from as far away as southern Germany, though the main portion of the seed came from local forests. Unfortunately, exact information on the seed source is lacking in many cases, but even so, these plantings have contributed much to a better understanding of the provenance problem. Striking differences with regard to straightness of bole, branching, growth vigour and resistance to mildew (*Microsphaera quercina*) have appeared in these plantings.

Good examples of improvement by selection are the so-called "Dutch oaks" (*Q. robur*). These are street trees in Holland grown from acorns of selected trees in the forests of Holland, probably near Breda. During nursery propagation a rigorous selection was made for straightness and growth vigour in order to obtain suitable street trees. The result is that acorns from these street trees give very fine plants for forest planting. Grown in dense stands in Denmark they have reached a height of 32 feet in 24 years.

Cieslar (16), in 1923, published the results of a 19-year-old provenance experiment with *Q. robur*. The experiment was made in Austria and represented 21

seed sources from widely different regions. The results strongly emphasize the importance of seed origin. This experiment will be discussed in more detail later.

Hauch (35, 36), in 1909, started a provenance experiment with *Q. robur* in Denmark at about Lat. 55° 30′ N., Long. 12° E. Four provenances from Denmark and one each from Holland, Russia (49° 30′ N., 34° 30′ E.), Gallicia, the mountains of northern Hungary, the lowland of southern Hungary, Czechoslovakia (48° 50′ N., 17° 20′ E.), Trieste, and northwestern Germany were included. In 1914 and 1920 he published results of this experiment. Striking differences in time of flushing and leaf fall and amount of summer shoots were observed. With regard to growth rate, provenances from Slavonia and Czechoslovakia proved the best. These regions are known to have some of the best oak forests in Europe. However, Hauch concluded that none of the foreign provenances was fitted for cultivation in Denmark because of susceptibility to early frosts and profuse development of summershoots which, in turn, caused heavy attack by mildew (*Microsphaera quercina*). Also, the southern provenances were characterized by longer but weaker annual shoots with poorly developed terminal buds.

Matthäi (57), in 1922, distinguished between six zones within the range of the European white oaks based upon time of flushing and length of the growth period—a purely phenological distinction.

Birner (8) reported that epicormic branch formation in *Q. robur* is a character which varies according to the racial type or variety. His experiment in Germany has lasted for more than 50 years and shows that the poorest oaks for timber production (i.e., those with many epicormic branches) come from the Elban district in Germany, while the best type for timber production (i.e., with few or no epicormic branches) come from certain regions in Saxony and from the Dutch border. The extensive ranges of many North American oak species indicate that the provenance problem is also a very important consideration for North American oaks. This is especially true when importation of acorns from North America is planned. Such introductions have been going on for some years, particularly with respect to *Q. borealis*. This species has proved very valuable and superior to any other oak for planting on sandy dry soils in northwestern Europe (7, 9, 53, 70). One of its good qualities is its soil-improving ability when planted in pine plantations (*Pinus mugo*) in the heath regions of western Denmark. The dead leaves are very quickly transformed into a good mull. Even where the top soil is raw humus over a hardpan, red oak is an effective soil improvement agent.

Selection of superior single trees has been underway for some time in Europe. In Sweden the "Society for the Promotion of Oak Cultivation" has been established (98). One of the purposes of this organization is the preservation of good oak trees by vegetative propagation on a large scale. The clonal lines thus established will be used for seed plantations. The demand for acorns in European forestry is in some countries far greater than the supply and thus justifies the establishment of seed plantations. Such plantations should, of course, be composed of many superior clones, and the variation in time of flowering between the selected clones must be considered. Also, the clones should be planted in alternating rows, or even mixed in the same row, to insure as many cross combinations as possible between the different clones. The presumed high degree of heterozygosity in most forest trees will probably, as mentioned by Langner (51), result in considerable segregation. For this reason seed of inferior genotype will doubtless also be harvested in such plantations.

In agricultural breeding it is essential that the plants to be used as a source of seed be as homozygous as possible with reference to the desired characters, since all the plants grown are harvested. The higher percentage of undesirable plants that segregate the less the profit. But in forestry only a relatively few plants are allowed to reach maturity, the greater percentage being removed by natural or artificial thinning, and consequently a high degree of genetic uniformity is not necessary.

The demand for acorns is, as mentioned before, rather acute in many European countries. In order to expedite seed production certain assumptions must be made with reference to the genotypic quality of the clones used. The clones can be selected only on the basis of the phenotype initially, since the quality of the genotype must be determined by progeny tests lasting several years. Such tests, preferably of control pollinated progeny, should be undertaken as soon as possible. Poor genotypes may then be discarded at an early date. By this means a seed plantation will never become static but will undergo constant improvement by selection. The seeds from such plantations should be used only in areas with climatic, edaphic and photoperiodic conditions similar to those existing in the locality where the parental trees were selected. For this reason several smaller local seed plantations are preferable to a few larger ones.

Controlled Hybridization

Relatively little controlled hybridization has been undertaken in *Quercus*. Flowering occurs at the time of flushing or a few days after unfolding of the leaves. The male flowers are born in pendulous catkins from the axils of the scales of the terminal bud or from special buds on one- or two-year-old wood. The female flowers, at least in North American oak species, are produced on shoots of the current season, mostly in the axils of the upper leaves, in solitary or many-flowered spikes. Some species are protogyneous (e.g., *Q. virginiana*), while others are protandrous (e.g., *Q. lanuginosa, petraea, robur, alba, macrocarpa*) (14). The male flowers are formed the previous season, while the females are formed during or just before the time of flushing. The ovules are formed very late and are not fully developed at the time of pollination. The ovary is three-celled, each cell containing two ovules, but only one of the six ovules in each flower is fertilized. Such reproductive characteristics make controlled pollination difficult. The small number of female flowers on each twig requires the use of many bags. Also, the time of receptivity seems to be rather short and to vary greatly from species to species, and the time interval between pollination and fertilization of the ovule is rather long. In the *Lepidobalanus* group the growth of the pollen tube follows an uninterrupted growth through the tissue of the style until the ovule is fertilized. In some species fertilization may occur nearly two months after pollination. But in the red oaks (Erythrobalanus), in which two years are required for maturation of acorns, the pollen tube stops growth when it has reached the base of the style. It then passes into a dormant or resting condition and remains there until the following spring when fertilization takes place.

Isolation of the female flowers can be effected by the use of glassine and kraft paper bags, the former inside the latter. Pollen should be secured from cut branches forced in a greenhouse. However, recent studies by the author indicate that oak branches do not force well if brought into the greenhouse too early. The catkins develop but the anthers may not open. In such cases the whole inflorescence wilts and dies. This is possibly due to the fouling of the conductive tissues by bacteria. Different

solutions have been used to keep the water clean but so far without success. Even repeated changing of the water and cutting of the bases of the branches are ineffective. The best method seems to be to start the forcing at the time of bud swelling or a little later. In this way the problem of storing the pollen for a longer period is avoided. If bagging of the female flowers is done early, the higher temperature in the bag will cause an earlier development of the female flowers than of the unbagged male flowers. This is advantageous because it allows removal of the bag at the time of pollination without danger of contamination by foreign pollen. Pollination can then be accomplished by applying the pollen onto the stigmas with a fine brush. More than one pollination is usually needed to insure fertilization. Later pollinations must be performed by injecting the pollen without removing the bag.

Storing of pollen can, according to Pjatnitskii (81), be achieved for at least 66 days or more when kept at low temperature (0° C.) in a desiccator containing a solution of 35% H_2SO_4. Germination tests of pollen can be made in a 0.2 to 1.6 *N* solution of sucrose in distilled water. For the sections *Cerris*, *Mesobalanus* and *Lepidobalanus* the best concentration was reported by Pjatnitskii to be a 0.4 to 0.6 *N* solution, while for *Q. borealis*, the best was a 1.0 *N*. Germination was greatest when some stimulator, such as a style or style extract, a 0.001% boron solution, folliculin or certain plant tissues were added to the nutrient medium. However, the fact that pollen germinates readily does not necessarily mean that it is capable of effecting fertilization of the egg. Pjatnitskii found that the female flowers are receptive for about a week. One of the most difficult artificial hybridization problems in oaks results from the irregularity of fruit set. For this reason trees showing regular and high percentage of fruit set should be selected for breeding work. Wright (110) has tried to treat the branches bearing young fruits with different chemicals to induce a heavier fruit set, but so far without positive results.

The best known artificially produced oak hybrids in North America are the Ness hybrids, produced in 1909 (68, 69). Helge Ness at the Texas Agriculture Experiment Station pollinated *Q. lyrata* with pollen from *Q. virginiana* and obtained three hybrid plants resembling the pollen parent. In 1910 he repeated the cross and obtained five hybrid plants. The hybrids show comparatively rapid growth, regular form and semi-evergreenness. In 1919 the hybrids flowered and set seed after open pollination. Twenty-three F_2 plants were obtained. Yarnell (111), in studying the variation in leaf size and shape in these F_2 plants, found that the plants fell into two distinct groups, one ranging around the *virginiana* parent, the other in a distribution below the *lyrata* parent, i.e., with leaves somewhat smaller than those of *Q. lyrata*.

In Russia S. S. Pjatnitskii has carried on rather extensive hybridization studies in *Quercus*. He even claims to have obtained hybrids between the two subgenera *Lepidobalanus* and *Erythrobalanus* (78, 79, 90) by pollinating *Q. robur* and *Q. macranthera* with pollen of *Q. borealis maxima*. The F_1's were reported to demonstrate vigorous growth the first year. The reciprocal crosses were unsuccessful, probably because of the biennial fruiting habit of species in the *Erythrobalanus* group. Pjatnitskii reported production of other hybrids, including the intersectional crosses *Q. macranthera* × *Q. robur* and *Q. macranthera* × *Q. alba*, both of which gave the highest yield of hybrid acorns among the 41 crosses he reported. Most of the hybrids showed intermediate characters and, to judge from the literature, some hybrid vigour. Crosses with cork oak

(*Q. suber*) were also attempted. Some of these have proved winter hardy in the northern part of the Ukrainian steppe. However, statistical data are not available with regard to the presumed hybrid vigour of some of Pjatnitskii's hybrids. Attempts to repeat some of his crosses are being made by the author.

Pjatnitskii also tried selfing of oak and reported (**77**) that many new and valuable characters may be revealed by this method.

Kolesnikov also tried selfing of oak and reported (**45*a***) that inbred oak seedlings at the age of one year were **8**% smaller than open pollinated seedlings, and that their coefficient of variability was one and a half times as great. At the age of two years the differences were much more pronounced, the difference in height amounting to **46.4**%, and the coefficient of variation was **2.2** times as great. Together with many small inferior plants, certain individuals considerably exceeded the maximum height for the normal plants (**57.1** cm. as compared with **42.0** cm.).

Schreiner and Duffield (**91**) found metaxenia (influence of pollen on maternal tissues of fruit) in a cross between *Q. alba* and *Q. robur*, some acorns being brown, as in the seed parent, others light green and late maturing, as in the pollen parent. They also found that hybrid acorns were larger than those produced by selfing.

Attempts at intraspecific hybridization apparently have not been reported in the literature. But with the increased interest in the establishment of seed orchards in Europe one may predict that intraspecific hybridization will be widely practiced.

Vegetative Propagation

Vegetative propagation in the oaks has been considered rather difficult, and this difficulty is said to be one of the reasons for the relatively little genetic research thus far undertaken in *Quercus*. Although oaks do root with difficulty, they are no more resistant to rooting than *Pinus* and *Picea*.

Thimann and Deslisle (**100**) found that it is impossible to root cuttings from older trees. They may survive for several months, but never become established. Dormant cuttings of three- to four-year-old plants, however, are relatively easy to root if treated in a solution of indolyl-acetic-acid. By using basal cuttings of three- to four-year-old plants taken in February, and by soaking them in a **400** p.p.m. solution of indolyl-acetic-acid for **24** hours, Thimann and Behnke-Rogers obtained **82**% rooting (**101**). Softwood cuttings taken during late summer from other species also rooted, but with a somewhat smaller percentage.

Grafting can be performed out-of-doors as well as in the greenhouse. For grafting in the greenhouse, two- to three-year-old potted plants should be moved into a temperate house in January or February. Established plants (i.e., plants potted the previous spring) are preferable. The grafting should be made when root activity starts a month or two later. Ordinary waxed whip grafts are the simplest and best. Quicker unions are obtained by keeping the grafts in a closed case with higher temperature and humidity, but this does not improve the percentage of take which, in recent experiments with grafting of young plants, is quite high (**90**%). The understock should preferably be of the same species as the scion, or at least from the same subgenus. However, it is possible to graft *Q. alba* on *Q. borealis*, that is, *Lepidobalanus* on *Erythrobalanus*. Mirov and Cumming (**59**) report that *Q. suber* (subgenus *Lepidobalanus*) can be grafted successfully on *Q. castaneaefolia*, *pubescens*, *iberica*, *petraea* and *chrysolepis*, all members of the same

subgenus; and also on *Q. kelloggii* and *douglasii* of the *Erythrobalanus* group. They reported *Q. suber* incompatible with *Q. agrifolia* and *Lithocarpus densiflora*.

Grafting in the nursery can be done in March or April when sapflow in the understock begins. In very dry and windy climates, however, it may be necessary to protect the scions from excessive desiccation by covering them with polyethylene bags over which should be put kraft paper bags to avoid excessive heating by insolation.

In order to make future pollination work easier it may be advantageous to graft scions on older, flowering, low and broad crowned trees so that the amount of climbing can be reduced. The grafts should be made on vigorous shoots which can be produced by heavy pruning of the tree to be used. Mirov and Cumming (**59**) report that cork oak can be grafted in this manner. The trees to be used for understocks are decapitated at a height of one and one-half to two meters, and the scions grafted the following year on the sprouts developing from just below the cut. In this way deer-browsing of the scions is avoided. They suggest that many of the chaparral oak species in the Southwest may be used this way as understocks for cork oak.

Pollinations in the greenhouse under more suitable conditions may be made by grafting old flowering wood on young potted plants, as is practiced in Sweden (**99**).

The introduction of grafting from horticulture into forestry thus not only enables forest tree breeders to preserve valuable genotypes but also makes the work of hybridization much easier.

Inducement of early flowering in oak has been reported from Sweden by Holger Jensen and Bertil Lindquist (**41**). *Q. robur* trees with a stem diameter of five centimeters and a height of about three meters were strangulated with a wire outside a narrow aluminum band. One of the trees thus treated yielded more than 1,000 acorns the following year.

Cytology

A number of chromosome counts have been made in *Quercus*. Cosens (**17**), in 1912, reported the $2n$ number to be 8. Wetzel (**108**), in 1928, found $n = 11$. Ghimpu (**32**), Hoegh (**39**), Sax (**88**), Jaretzky (**40**) and Vignoli (**105**) found the haploid number to be 12, while Friesner (**30**) and Aufderheide (**5**) reported it to be 6. Later counts (**23**, **29**, **67**) gave the haploid number as 12 which is now considered the basic number for the genus *Quercus*. Natividade, however, found that the chromosomes are made up of 12 types and that the same types could be recognized in eight European species and two hybrids. He found that the 12 types could be further divided into two groups of six, differing only in size. He also observed secondary associations at the first and second metaphase. This phenomenon he interprets as evidence of a possible basic number of six. One to three chiasmata were observed, according to the size of chromosomes. Hoegh (**39**) noted irregularities during meiosis in the pollen mother cells from a presumed natural hybrid between *Q. robur* and *Q. petraea*. Univalents were found during anaphase. He observed that meiosis starts three to four days before leafing, i.e., when the male flowers are still enclosed in the swelling buds. The divisions lasted eight to ten days.

Helge Johnson reported (**42**) on polyploidy in twin seedlings of *Q. robur*. Out of about 75,000 acorns he obtained 314 twin pairs and 31 triplets. Chromosome counts of these plants gave a frequency of 0.41% triploid. Naturally occurring triploid adult trees have, however, never been reported, in spite of rather intensive cytological investiga-

tions by several workers in Sweden. Helge Johnson is therefore of the opinion that such triploid oak twins probably originate from abnormal embryos. Machado in 1943 (56) reported that colchicine treatment of *Q. suber* resulted in various modifications in the cells but did not alter the number of chromosomes.

Studies of Inheritance

Many European data have been published on the correlation of various characteristics in oaks. In Austria, Cieslar (16) planted acorns of 21 trees from different parts of Europe. The mother trees were described as to form and growth rate. He found a correlation between mother tree and progeny in these qualities. He also observed a correlation in *Q. robur* between width of crown, straightness of bole and growth rate. Narrow crowned trees with ascending branches were generally faster growing. However, caution should be taken in judging the degree of genetical control of form when the trees are grown under climatic and photoperiodic conditions widely different from those existing at their place of origin. Cieslar found that acorn size influenced the height growth of seedlings until at least their seventh year. He gives the following data:

TABLE I

Weight/1000 acorns (grams)	Height at various ages					
	1 year		7 years		18 years	
	cm.	%	cm.	%	cm.	%
7670–5090	22.7	100	132.9	100	497	100
4820–4040	17.1	75	132.9	84	499	100
3770–1320	15.3	67	105.6	79	512	103

After 18 years of growth the differences in height disappeared completely. Eitingen (25) found that a proportion of 100:65:31 in acorn size gave a correlated proportion of 100:95:76 in height of eight-year-old plants. Helge Johnson (43) reported that differences in height due to acorn size existed even in 18-year-old plants.

Acorn size also influences the percentage of germination. Helge Johnson (43) gives the following data:

TABLE II

Weight per 1000 acorns (kg.) ...	9.20	5.34	3.94	3.12	2.12
Number of plants per 100 acorns..	125	79	94	89	73

It may be surprising that 125 plants were derived from 100 acorns, a fact attributable to the large acorns often containing two embryos. As many as six viable embryos may be found in one acorn (11, 21).

The effect of acorn size on growth rate must therefore be taken into consideration when studies of the genetical control of growth rate are undertaken. If great differences in acorn size exist, determination of comparative vigour must either be delayed until the plants are ten to 15 years old, or the acorns may be segregated into size classes at the time of planting. By this means differences within the size classes may be determined much earlier.

In 1911 Oppermann (73) initiated a detailed progeny study of several trees of *Q. robur* in Denmark. He came to the conclusion that there is a fairly high degree of positive correlation between the form of the mother tree and that of its progeny. His data follow:

TABLE III

	All very good	Both good and bad	Mostly poor
Progenies of good mother trees (13 progenies of individual trees and 15 of stands)	61%	25%	14%
Progenies of good mother trees (28 progenies of individual trees and of 6 stands)	15%	26%	59%

It will be seen that the majority of the progenies of good trees resembled their mothers, and the same was the case with progenies of poor trees.

Krahl-Urban (**46**), by studying natural regeneration of oaks in Germany, concluded that there were similarities in shape of crown between the presumed parents and their progeny.

Genetical differences between different progenies can be detected only if the progenies are grown together under uniform environmental conditions. However, the phenotypic expression of such differences may be considerably modified in a different environment. Therefore caution should be taken when considering the suitability of a certain progeny to be grown under environmental conditions different from those under which the progeny test was made.

There is some evidence that crown form in some oaks is inherited in a simple Mendelian manner. A pendulous form of *Q. robur*, the so-called Burana oak of southern Sweden, is a good example. It was discovered in 1850. Since then three open pollinated generations have been grown, and in each pendulous forms have appeared (**73**). Pjatnitskii (**82**) concluded from a progeny test of a specimen of the fastigiate form of *Q. robur* that the fastigiate habit is dominant and that the particular tree tested was heterozygous with respect to this character. However, no data on the quantitative result of these two experiments are available. Esson (**27**) planted three acorns from the fastigiate form of the same species and obtained two normal and one fastigiate plant. In this connection it is of interest to note that about 150 years ago in Germany, the form of a pyramidal oak was attributed to the fact that it grew in an old abandoned well.

Funk reported (**31**) on variegation in *Q. robur*. The progeny of a white variegated tree contained 29% variegated seedlings, among which all degrees from nearly pure green to white occurred, the growth rate being greatly reduced with increased variegation. The summer shoots developed by these seedlings had less variegated leaves than those developed during the spring. This is doubtless a case of "albo-maculatus" variegation, in which transmission of the variegation is effected through the cytoplasm.

A few cases of albinism have been observed by the author among seedlings of *Q. borealis*. The plants died at the age of four to five months.

Time of flushing seems to be determined rather rigidly by the genotype. Cieslar (**16**) mentions a so-called variety of *Q. robur* from the Save River Valley in Yugoslavia which flushes two weeks later than other oaks of the same species (*Q. robur* v. *tardissima*). Such diversity is characteristic of geographical races, and to give such variants varietal status seems unnecessary. Of peculiar interest is the variation in time of flushing often found inside rather small populations. In Sweden Sylven (**97**) made observations over a period of three years on the time of flushing and leaf fall among more than 300 *Q. robur* trees of a single population. He found considerable diversity from tree to tree. Generally the same relative time of flushing was recorded each year for each tree, but some fluctuations from year to year were noted. The variation from tree to tree within the same population is of great importance to forest tree breeders. If southern races are moved northward it would be advantageous to select late flushing and early leaf shedding types in order to avoid late spring or early autumn frost damage. However, they should not be moved so far north that the day length becomes much greater than at their place of origin. Under such conditions the growth period in autumn will be prolonged and may result in damage by early fall frosts. However, movement north may be con-

siderable if the growing season in the new habitat is long enough to allow for a prolonged growth period in autumn.

Racial Differentiation

Many oak species have a very wide natural distribution. *Q. borealis* and its variety *maxima*, for example, is found from Nova Scotia to Florida and west to Minnesota and Texas. Racial differentiation may thus be assumed. Intraspecific differences with regard to

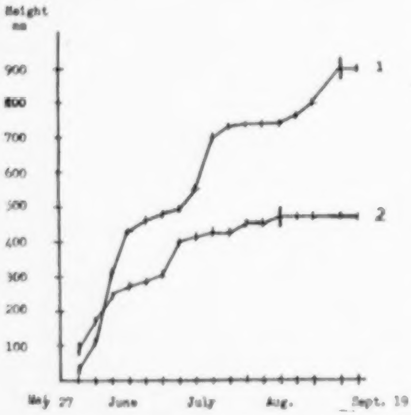

FIG. 1. Graphic illustration of the growth during one summer (1952) of *Q. borealis* from a northern and a southern source when grown at Boston. 1: From Blairsville, Georgia, at Lat. 44° 52′ N. and Long. 83° 58′ E. Average of six plants. 2: From Madison, Maine, at Lat. 44° 52′ N. and Long. 68° 41′ E. Average of seven plants. See text for further explanation.

morphology and physiology are common in many oak species. Although the morphological differences are the easiest detectable, they probably are of less significance than the physiological differences. Cieslar (16) has investigated the frequency of summershoot formation in different races in *Q. robur* when grown at Wienerwald, Austria. He concluded that races with many summershoots are those from mild regions with a high precipitation during the summer months. Such races were heavily attacked by mildew (*Microsphaera quercina*).

Recent experiments, carried out by the Maria Moors Cabot Foundation for Botanical Research of Harvard University, have shown that some races, especially those of extreme southern origin, produced two shoot elongations during one summer, i.e., three elongation periods per season, if the spring elongation be included. In Fig. 1 is shown the growth of a northern and a southern race of *Q. borealis*. Only one season's growth is shown, based on weekly measurements. Note that the southern race has three elongation periods while the northern has only two. On the average they show 2.7 and 2.1 elongation periods, respectively. Also, there is a three-week difference in time of height growth cessation. Both of these phenomena are probably due to the different photoperiodic reactions of the two races. It is well known in other tree species that when the day is prolonged for different races, either artificially or by moving them northward, their period of growth is lengthened. In some cases damage by early autumn frosts may occur. Moschkov (60) found that southern races of *Salix babylonica*, *Salix pierottii*, *Rubus idaeus* and *Vitis amurensis* moved to Leningrad could be made hardy there simply by shortening the day artificially. Only a treatment of 20 days was required for some plants to enable them to stop growth. He found that short-day treatment of only the growing tip of the shoots was enough. Untreated plants were heavily frost damaged.

With regard to *Q. borealis*, Kramer (47) claims that they do not continue growth late in fall when treated with continuous light. However, continuous light may have a different effect on the plants than just a longer day. Some of the races of red oak grown by the Cabot Foundation near Boston (42° N. Lat.) have also been planted near Co-

penhagen, Denmark (55° N. Lat.). While the winters in these two localities are of about the same severity, but tending to be more continental at Boston, the difference in day length is considerable. This may be the explanation of the fact that about 30% of the plants of Georgian origin (55° N. Lat.) were killed by the first winter in Denmark, while at Boston plants from the same seedlot suffered no, or only very slight, frost damage. Recent, but as yet incomplete, experiments with *Q. borealis* plants treated with artificially prolonged day strongly indicate that such treatment prolongs the active period of growth. Southern races when grown at northern latitudes, or when treated with artificially prolonged days, therefore show a greater seasonal height growth due to the prolonged period of active growth. In Fig. 2 the growth rate at Boston of one-year-old seedlings from acorns of different latitudes is illustrated graphically. The southern races are generally taller than the northern ones. Exceptions to this rule do, of course, occur which may be traceable to seed size and other factors.

Fig. 3 shows the relation between time of flushing and latitude of origin when grown at Boston. It will be seen that plants of northern origin are the earliest

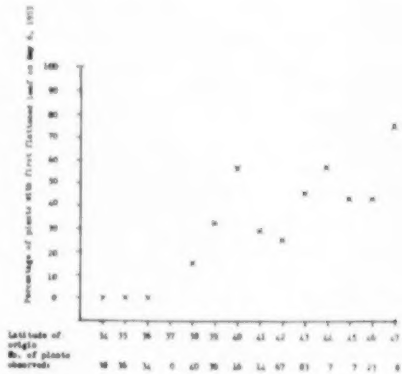

FIG. 3. Correlation between time of flushing and latitude of origin when grown at Boston.

flushing, while those of southern origin are late.

Cieslar (16) reported that continental races of oak have a more colorful and earlier leaf fall than maritime and southern races. This has also been observed in red oaks and is probably common in all deciduous trees occupying a wide natural range.

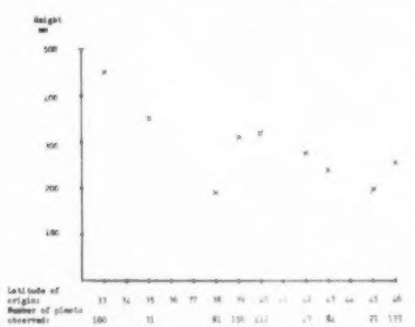

FIG. 2. Correlation between latitude of origin and height growth of one-year-old seedlings grown at Boston, 1952.

Summary

The genus *Quercus* is represented by about 300 species in the north temperate zone, the American Tropics to Colombia and the Malayan Archipelago. Classification and nomenclature are very confusing because of great intraspecific variation in the genus. Many of the European species have been divided into numerous varieties and forms, some of which are probably of doubtful taxonomic rank. The naming of presumed natural hybrids has also added to the confusion. Natural hybridization within the sections is known to occur, but the frequency of hybrids in wild populations is low. In spite of the fact that most hybrids are fertile, hybrid swarms are relatively rare The role played by hybridization in speciation is difficult to assess due to the long time span between

generations. Hybridity often occurs between allopatric species in zones of range overlap, while hybridization between sympatric species may be rare.

Selection from wild stock has apparently been undertaken on a very small scale in the United States and Canada. Selection of phenotypically superior trees and progeny tests of such trees is underway in Europe with the objective of establishing seed plantations.

As a result of the irregular flowering habits of most trees and because of various technical difficulties, controlled interspecific hybridization has been undertaken only on a relatively small scale. The hybrids produced by Ness in Texas and by Pjatnitskii in Russia seem to indicate, however, that interspecific hybrids may prove valuable. Future forest-tree improvement in *Quercus* will probably be concerned mainly with selection from wild stock and with intraspecific hybridization.

Vegetative propagation has been considered rather difficult. Cuttings from older trees are impossible to root, but the basal part of three- to four-year-old plants have been rooted with success. Grafting presents no major problems and can be done as well in the greenhouse as out-of-doors. Inducement of early flowering by ringing or strangulation appears promising.

The diploid chromosome number is now considered to be 24 in all *Quercus* species investigated. Irregularities in meiosis have been found in plants of hybrid origin.

Progeny tests in Europe give evidence that such characters as form, growth rate and type of photoperiodic reaction are strongly controlled by heredity. Simple Mendelian inheritance of certain traits is known.

Racial differentiation is common in many species with a wide range, and there is evidence that physiological variation is quite as common as morphological variation. Production of summer shoots and continuation of growth in fall as photoperiodic reactions is discussed in connection with results of recent experiments.

Acknowledgment

Grateful acknowledgement is made to the Maria Moors Cabot Foundation for Botanical Research, Harvard University, for its support of research in this field, and to Dr. Scott S. Pauley, A. G. Johnson and E. J. Palmer for their valuable suggestions and criticism.

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Utilization Abstracts

Naringin. Grapefruit (*Citrus paradisi*) and shaddock, or pummelo (*C. grandis*), are distinguished from other citrus fruits, among other characters, by their containing the bitter glucoside naringin which is largely responsible for the bitter flavor in this fruit. It has been extracted and sold commercially in small amounts under the name "Amerin" for use in flavoring beverages and confections. Possible other uses awaiting development and exploitation are as an organic chemical in the preparation of acid azo dyes and wood stains, vitamin P, rhamnose, coumaric acid and phloroglucin. (J. W. Kesterton and R. Hendrickson, *Fla. Agr. Exp. Sta., Bull. 511. 1953*).

Huon Pine Wood Oil. The wood of Huon pine (*Dacrydium franklinii*), a Tasmanian timber tree, contains 90 to 95% methyl eugenol, and for about 20 years waste wood and shavings of this species from lumber mills constituted the commercial source of the oil, unsurpassed for exterminating powder post and furniture borers and as a preservative for water paints and similar preparations based on casein. Because of increasing inaccessibility of the trees, this oil is no longer on the market. The most promising Australian substitute at present is the oil of *Melaleuca bracteata* with 60 to 90% ethyl eugenol. (A. R. Penfold, *Austral. Jour. Pharm. 33: 851. 1952*).

Dehydrated Alfalfa Prevents Scale in Sea Water Evaporators

The most economical method of obtaining potable water from sea water is distillation. This method would be more economical if scale formation in evaporators could be eliminated. The present article suggests a completely new and different approach to the problem of eliminating scale in sea water evaporators.

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When hard water or sea water is evaporated in an evaporator to produce distilled water, it is a well known fact that the instrument soon becomes coated with scale. Industry tries to minimize this nuisance by " blowing down " or rejecting one half of the residual heated water and in that manner getting rid of the scale-forming chemicals. This " blow down " system does not entirely eliminate the scaling of evaporators.

It was noted that if pulverized dehydrated alfalfa was added to water to be distilled, no scale lined the evaporator. A series of experiments showed that if 0.1% of dehydrated alfalfa was added to the water, scaling could be prevented.

This was first tested with two evaporators in Waukesha County, Wisconsin, using the very hard water found in that locality. The two evaporators were operated continuously for 72 hours; one was fed the hard water, the other was fed the hard water with 0.1% of dehydrated alfalfa. The 50% " blow down " principle was used with these evaporators. At the end of 72 hours the instrument fed the plain hard water was found to be heavily scaled, while the one that was fed the water containing the alfalfa was scale-free.

Plant foliage is not commonly used as a boiler compound or as an anti-scale compound, as described in this paper. The patents that have been issued since 1860 show that only one claims that dehydrated plant foliage can prevent scale. In a patent specification, dated January 14, 1890, Erastus C. Noland claimed " that a mixture of wheat straw, wheat chaff and thrashed wheat, if placed in a porous sack and hung in a boiler, would not only prevent scale, but would effectually remove the burned iron or incrustation from the boiler walls " (1).

The successful manner in which alfalfa prevented formation of scale in producing distilled water from hard ground water led to the evaporating of sea water in these same evaporators. One was fed sea water, while the other was supplied with sea water with added alfalfa. After a 144-hour run the evaporators were shut down and dismantled. Upon inspection the evaporator supplied with plain sea water was heavily scaled, while the evaporator using water treated with alfalfa showed no evidence of scale.

The problem that presented itself was to find the component or group of components present in alfalfa that prevented formation of scale when water with a large amount of dissolved mineral matter was distilled with a 50% blowdown system during the distillation.

The quantitative experiments used to prove the scale-preventing properties of the various components of alfalfa were very simple, yet conclusive. A description of the experimental procedure follows:

A numbered plate of Admiralty metal, an alloy of 70% copper, 29% zinc and 1% tin, commonly used in sea water evaporators, was bent into a — shape. Before bending, the dimensions of the plate were 6.5 by 10.5 cm. The plate was cleaned with acid, washed with distilled water and boiled repeatedly in distilled water until its dry weight became constant within two tenths of a milligram. The plate was then placed in a 1000-ml. beaker in such a manner that the wide surface of the plate was not in contact with the bottom of the beaker. 700 ml. of sea water were put in the beaker. The sea water used was artificial, since the natural sea water available was greatly contaminated with iron hydroxide. The formula of Brujewicz (Subow 1931) was used (2).

The beaker was heated until 350 ml. of water had evaporated, the remaining water was siphoned off and a fresh volume of 700 ml. of sea water was added. In this way was the 50% blow down simulated. Ten boilings of this kind were conducted for each test. At the end of ten boilings, the plate was removed, washed with distilled water, dried and weighed. Any gain in weight would be the scale formed while 7000 ml. of sea water had been concentrated to 3500 ml. Any loss in weight would be due to corrosion of the metal. In the rest of this paper this method will be referred to as the ten boiling scale cycle.

Treatment of the sea water for the various tests was made to simulate reported conditions with regard to dehydrated alfalfa. To secure a 0.1% concentration, 0.7 g. of alfalfa or an amount of a derivative of alfalfa equivalent to 0.7 g. of alfalfa was added to each 700 ml. of sea water.

To determine the active component of alfalfa various hypotheses were made initially and these hypotheses were then tested.

Hypothesis I

Dehydrated alfalfa is of no value in preventing scale formation.

This hypothesis was tested to settle any questions concerning the claims for alfalfa based on the work completed on the two evaporators in Waukesha County mentioned previously. Ten cycles of evaporation were conducted on sea water without treatment with alfalfa, and ten cycles with sea water having a 0.1% concentration of alfalfa.

Plate 5 was used with untreated sea water; plate 6 with sea water containing 0.1% powdered alfalfa.

Plate No.	Original Weight	Final Weight	Result
5	59.1087 g.	59.1441 g.	0.0354 g. scale formed
6	74.9923 g.	74.9796 g.	0.0127 g. loss of metal

Hypothesis rejected.

Hypothesis II

Addition of 0.1% alfalfa lowers the pH of sea water to such an extent that deposition of scale is impossible.

To answer this the pH of the solutions used in testing hypothesis I was measured at various times with the following results:

a) Original sea water, pH 7.2 (subsequent tests on other samples of untreated sea water showed that the pH varied from 6.8 to 8.3).

b) Sea water evaporated to half volume, pH 8.2 (subsequent evaporations showed that this value varied from 7.9 to 8.2).

c) Sea water with the alfalfa, pH 6.6, this water evaporated to half volume, pH 7.8.

A value only slightly below 7 does not indicate any practical acidity so that the value of 6.6 for the alfalfa-treated water is not significant. Further, subsequent tests showed that a pH of 6.5 is not effective in preventing scale.

Hypothesis rejected.

Hypothesis III

THE VALUE OF ALFALFA IS DUE TO ITS MINERAL CONTENT.

200 grams of alfalfa were ashed, yielding 14.5 grams of mineral substance, indicating a 7.25% ash content. Hence 0.0508 gram of ash are equivalent to 0.7 gram of alfalfa. This amount of ash was added to each 700 ml. of sea water used in the test. Ten cycles of evaporation were undertaken. The scale was so heavy after five cycles that evaporation was discontinued. Since the ash is alkaline, the pH of the treated water before evaporation was slightly alkaline, though not seriously. Before evaporation the pH was 8.1; after evaporation to half volume, 8.7.

Hypothesis rejected.

Hypothesis IV

THE VALUE OF ALFALFA IS DUE TO AN OIL OR OIL-LIKE SUBSTANCE WHICH PREVENTS THE SEA WATER FROM DEPOSITING.

25 grams of alfalfa were placed in a closed container and soaked with 200 ml. of ethyl ether. After 24 hours, the extract was decanted and diluted to 360 ml. with more ethyl ether. Thus 10 ml. were equivalent to 0.7 gram of alfalfa. This was a dark green liquid and contained, besides chlorophyll, any oils or fatty organic substances present in the alfalfa. In addition, certain other components may have been extracted. The residue from the extraction was dried and saved; it was a green powdery material. Boiling cycles were conducted; in one instance the sea water was treated with 10 ml. of the ether solution, in the other with 0.7 gram of the green extracted alfalfa.

Plate 0 was used in the water treated with the ether solution, while Plate 1 was used in the water treated with 0.7 gram of the extracted alfalfa residue.

Plate No.	Original Weight	Final Weight	Result
0	71.6221 g.	71.7455 g.	0.1234 g. scale formed
1	71.0122 g.	70.9824 g.	0.0298 g. loss of metal

Hypothesis rejected.

Hypothesis V

THE VALUE OF ALFALFA IS DUE TO A SUBSTANCE WHICH IS A COMBINATION OF MINERAL AND ORGANIC PARTS, PROBABLY OF THE NATURE OF A SOAP.

25 grams of alfalfa were placed in a closed container with 200 ml. of 95% ethyl alcohol. The residue was dried and saved; it was a gray-green powder. The alcohol solution was a light green liquid. The boiling cycles were conducted; in one set the sea water was treated with 10 ml. of the alcohol-extracted alfalfa.

Plate 8 was used in the sea water treated with the alcohol solution, while Plate 9 was used in the water treated with the extracted alfalfa.

Plate No.	Original Weight	Final Weight	Result
8	57.1087 g.	57.3725 g.	0.2638 g. scale formed
9	74.9920 g.	74.9893 g.	0.0027 g. loss of metal

Hypothesis rejected.

Hypothesis VI

THE VALUE OF ALFALFA IS DUE TO SUBSTANCES WHICH ARE ESSENTIALLY INSOLU-

BLE IN TYPICAL ORGANIC SOLVENTS BUT WHICH ARE SOLUBLE IN WATER.

The first part of the hypothesis was proven by the results of tests under hypotheses 4 and 5. 25 grams of alfalfa were placed in a closed container with 200 ml. of distilled water and stored at 60° C. for 24 hours. The extract was decanted and diluted to 360 ml. with water. The residue was dried and saved; it was a green powder. The water extract was a light brown liquid. The boiling cycles were conducted; in one set the sea water was treated with 10 ml. of the water extract and in the other the sea water was treated with 0.7 gram of the extracted alfalfa.

Plate 10 was used in untreated sea water; plate 19, with sea water containing 0.1% of water extracted alfalfa; plate 13, with sea water treated with 10 ml. of aqueous extracts of alfalfa.

Plate No.	Original Weight	Final Weight	Result
10	74.5909 g.	74.6329 g.	0.0420 g. scale formed
19	72.6736 g.	72.6566 g.	0.0170 g. loss of metal
13	71.6440 g.	71.6894 g.	0.0454 g. scale formed

Hypothesis rejected.

Hypothesis VII

THE VALUE OF ALFALFA IS DUE TO ITS WATER-SOLUBLE INGREDIENTS AND THEIR COLLOIDAL ACTION.

Hypothesis VIII

THE VALUE OF ALFALFA IS DUE TO THE MECHANICAL ACTION AND ABRASIVE ACTION OF SUSPENDED FIBERS.

Plate 11 was used with sea water treated with 0.7 g. of alfalfa encased in a porous plastic bag to prevent any fibrous abrasive action on the metal. Plate 7 was used in sea water treated with the 0.7 g. of alfalfa encased in a collodion bag to eliminate colloidal action.

Plate No.	Original Weight	Final Weight	Result
11	70.5430 g.	70.5336 g.	0.0094 g. loss of metal
7	72.7770 g.	72.7702 g.	0.0068 g. loss of metal

It is readily seen that abrasion and colloidal action play no part or, at most, a very small part in preventing scale. The fact that the amounts of metal loss were smaller than usual can be explained by the fact that the bags, used to encase the alfalfa, floated on top of the boiling water; this prevented good contact between the alfalfa and the water. Less perfect extraction of active component resulted.

It is further seen that the active components of alfalfa must be soluble in salt solutions, in slightly basic salt solutions (since sea water is alkaline).

Last part of Hypothesis 7 rejected.

Hypothesis 8 rejected.

To check the first part of hypothesis VII, two 10% solutions of sodium chloride were made up. Solution #1 had a pH of 7.0; solution #2 was adjusted to a pH of 8.3. These solutions were used to extract alfalfa at a temperature of 100° C. The amount of extract used in each case was such as to give an effective concentration equal to a 0.1% concentration of raw alfalfa. Plate 8 was used in the sea water treated with the neutral salt water extract of alfalfa; plate 16 was used in the sea water treated with the basic extract.

Plate No.	Original Weight	Final Weight	Result
16	75.4487 g.	75.4578 g.	0.0091 g. scale formed
8	73.8807 g.	73.8872 g.	0.0065 g. scale formed

Although there was no loss of metal when the saline extracts were used, as there was when raw alfalfa was used,

the active factor seems to be salt-soluble, since the weights of the scale formed, 9.1 and 6.5 mg., compare favorably with the weights of scale formed by untreated sea water (35.4 and 42 mg.). The fact that this small amount of scale formed can be ascribed to incomplete extraction of the active ingredient from the alfalfa.

The fact that the anti-scale factor was soluble in neutral salt solution might be an indication that it is a protein or a polypeptide of the globulin class. Further tests supported this supposition. For example, a light brown amorphous solid was precipitated from the neutral salt solution by adding alcohol. The salts of the heavy metals also caused precipitation, forming a dark yellow amorphous solid which was probably the metal proteinate. The precipitate formed by the alcohol had no melting point and was insoluble in water and organic solvents. It could be redissolved in a 5% salt solution, only with difficulty, indicating that denaturation took place during coagulation.

It was desired to test the material obtained from the salt solution extract of alfalfa by precipitation with alcohol, in spite of the fact that the protein seemed to have been slightly denatured in the process of obtaining it. Accordingly, alfalfa was extracted with hot 10% sodium chloride solution; the extract was allowed to cool and an equal volume of ethyl alcohol was added. The gelatinous precipitate thus obtained was separated from the mother liquor by centrifugation. It was dried in a desiccator, and five grams were placed in 200 ml. of 10% sodium chloride solution. The material was only slightly soluble, even upon prolonged standing. The anti-scale properties of this solution were tested in the usual boiling cycles. 20 ml. of the solution, along with the suspended particles of undissolved material, were added to 700 ml. of sea water.

Plate No.	Original Weight	Final Weight	Result
9	69.3217 g.	69.3271 g.	0.0059 g. scale formed

In view of the small amount of scale formed (5.9 mg. compared to 42 mg. and 35.4 mg. from untreated sea water), it was concluded that this material obtained might be the anti-scale substance of alfalfa, but its properties had been somewhat lessened by the action of the alcohol used to bring about its isolation.

Results of both the qualitative and quantitative tests with alfalfa indicated that only a small amount of the active component was necessary to afford scale prevention and that a concentration of 0.1% alfalfa was far in excess of requirements. As a preliminary test of the extended activity of alfalfa before exhaustion, it was decided to conduct boiling tests in which 350 ml. of additional sea water would be added to the 350 ml. alfalfa-containing residue and evaporated to half volume, this to be continued until a noticeable scale had formed. Accordingly 700 ml. of sea water containing 0.7 g. of alfalfa were evaporated to 350 ml. and observed for scale. Then an additional 350 ml. of untreated sea water was added and the evaporation repeated. Results showed that no noticeable scale had formed until the fifth addition of sea water had been evaporated. During the last evaporation a heavy deposit of scale suddenly formed. This indicated that, even without blowdown, a concentration of raw alfalfa as low as 0.035% was effective in preventing scale.

Conclusions

1. Sea water deposits scale when it is evaporated in an evaporator with a 50% blowdown.

2. Addition of 0.1% of dehydrated alfalfa to sea water will prevent forma-

tion of scale in an evaporator with a 50% blowdown.

3. Addition of 0.1% alfalfa does not greatly alter the pH of sea water; therefore, this cannot be used to explain the prevention of scale.

4. The ash of alfalfa does not prevent formation of scale.

5. The ether-soluble portion of alfalfa does not prevent scale formation.

6. The alcohol-soluble portion of alfalfa does not prevent scale formation.

7. The ingredients of alfalfa soluble in distilled water do not prevent scale.

8. The ingredients of alfalfa soluble in neutral saline solution and in alkaline saline solution do prevent scale formation but not to the same extent as does raw alfalfa.

9. The prevention of scale is not caused by the abrasive action of the fibers of the plant or by any colloidal action.

10. The alcohol-precipitated material from a neutral saline extract of alfalfa does possess scale-preventing properties.

11. A concentration as low as 0.035% of alfalfa with no 50% blowdown will prevent formation of scale when sea water is distilled.

Literature Cited

1. Noland, E. C. U. S. Patent 419470, Jan. 14, 1890.
2. Sverdup, H. V., Johnson, M. W., and Fleming, R. H. The oceans. 1946. [p. 186].

Utilization Abstract

African Oil Palm. It has been estimated that a 34% increase in world production of vegetable oils and fats will be needed to meet the demands of increased population by the year 1960. One of the most promising and as yet relatively unexploited sources of such oils is the African oil palm (*Elaeis guineensis*) which, under favorable conditions, yields up to the almost fantastic total of two tons of oil per acre. This palm grows naturally in tropical Africa from the French colony of Senegal to the Portuguese colony of Angola, especially in the coastal belt of 100–150 miles depth from Sierra Leone to the Cameroons. In this region palm oil is an important part of the African's diet, consumption of it in this respect amounting to 100 pounds per head per annum in some districts of Nigeria. Exports of the oil from West Africa constitute a true surplus, available only after domestic requirements have been satisfied. Only a little of the crop is harvested from plantations; most of it comes from wild trees; and in the Belgian Congo alone, in 1951, production of the oil amounted to 200,000 tons. In 1910 the palm was introduced into the Far East, and since then plantations there have been developed to such a degree that between that year and 1940, combined annual exports of the oil from Sumatra and Malaya grew from zero to nearly 300,000 tons.

Numerous varieties of *Elaeis guineensis* have been described, differing principally in the thickness of the kernel shell which is surrounded, when the fruit is ripe, by the oil-containing and comparatively soft pericarp. On this basis the main forms are distinguished as varieties *dura*, *macrocarya*, *pisifera* and *tenera*.

In West Africa extraction of the oil consists of first boiling and pulping the fruits, then pressing the oil out of the pulp and finally removing the nuts from the fibrous material. On the modern large plantations in Malaya, Indonesia and the Belgian Congo, where up to 20,000 tons of fruit may be handled per year, the sequence of operations is as follows: *a*) sterilization of the fruit branches, *b*) mechanical separation of fruits from the bunches, *c*) digestion of the fruit, *d*) separation of the oil in a centrifuge or press, *e*) washing the crude oil, *f*) purification of the washed oil, *g*) separation of nuts and pericarp residues, *h*) drying the nuts, *i*) cracking the nuts, *j*) separation of kernels and fragments of shell, *k*) drying the kernels. Utilization of kernels is less profitable than that of oil from the pulp. (Miss R. M. Johnson and W. D. Raymond, *Colonial Plant and Animal Products 4(1): 14. 1954*).

Cultivation of Peppermint in Florida[1]

In this comparative study of cultural, morphological and histological characteristics of species of Mentha growing in that State, it is concluded that extensive cultivation of the herb there would not be commercially profitable.

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Introduction

Peppermint is the most important among cultivated medicinal and aromatic plants used in the drug, food and cosmetic industries if total acreage under cultivation throughout the world is regarded as the sole criterion of importance. In the United States the herb has long been cultivated on a commercial scale in Oregon, Washington, Michigan and Indiana [2], and many persons have wondered whether this plant, which grows so profusely in those States, might not make even better growth in the year-round milder climate of Florida, and whether it might not become a commercially profitable crop for that State. Results obtained from greenhouse cultivation and field trials, as well as experiences reported by others, now permit a fairly definite answer in the negative to this question and are presented in this article.

[1] This article, the fourth in a series on Florida mints, is based on research with the College of Pharmacy, University of Florida, Gainesville, and on a paper presented before the Florida Academy of Sciences, Annual Meeting, Nov. 21, 1941. That paper has been thoroughly revised for publication here.

[1a] Deceased Oct. 28, 1954.

[2] Considerable data on the oils from these States and from Ohio and California are contained in the article by Ellis and Stevenson (9).

World Production

In 1930, 1,272,000 kg. (2,804,760 lbs.) of crude peppermint (i.e., the singly distilled volatile oil, which is the chief commercial product) were produced throughout the world on a cultivated area estimated as at least 110,000 acres, assuming an average of about 25 lbs. of oil per acre per annum (28). Of this amount, the United States furnished approximately one-third (400,000 kg. or 882,000 lbs.), grown on about 40,000 acres [3]. The latest figures available for world production of peppermint oil are for the year 1938 [4]. They indicate a total production of 1,328,500 lbs., of which the U.S.A. furnished 55.1%; Soviet Russia, 24.8%; Bulgaria, 10.8%; and the balance (9.3%) was divided among Italy, China, Hungary, Roumania, England, France and other countries. The figure for 1930 is higher because it includes the very

[3] In Germany, a few years back, species of *Mentha* (especially peppermint) occupied about 26% of the total area devoted to medicinal and spice plant cultivation (5).

[4] An effort was made to obtain more recent figures on world production of peppermint oil, but the U. S. Departments of Agriculture and Commerce, the Food and Agriculture Organization of the United Nations, as well as Fritzsche Brothers and other commercial firms were all unable to provide these data. This deficiency of intelligence appears rather amazing in view of the large size of this industry.

large Japanese peppermint oil production.

There are indications that subsequent production quantities are considerably greater than those for 1938. Thus Argentina produced 44,092 lbs. in 1947; Brazil, 2,200 lbs. ca. 1943; Italy, up to 110,000 lbs. in recent years; Morocco, 11,000 lbs. in 1946; Roumania, 22,000 lbs. in 1940; while in 1946 the U.S.A. far exceeded the total world production of 1938 by bringing forth in that year 1,620,000 lbs. (15) Total production of the U.S.A., Italy and Roumania in these later years was up to 1,752,000 lbs., whereas the same countries in 1938 produced only 816,700 lbs. It is quite possible that total world production today is double that of 1938 [5].

Geographical Distribution of Cultivation

Peppermint is grown over a considerable range of latitude. Thus the plant has been reported to thrive in the Leningrad area of the Soviet Union (22) and almost anywhere in the coastal regions of Alaska (12), both regions being at about 60° N. Lat. With suitable provision for shelter and exposure to sunlight, peppermint can be successfully produced even in interior Alaska, almost to the Arctic Circle at 66.5° N. Lat. (12). At the other extreme, successful cultivation of this plant has been reported in Kenya, East Africa, on the equator (23); and details of cultivation near Bangalore, India (13° N. Lat.), are available (25).

Although it is beyond the scope of this paper to discuss yields and qualities of peppermint oils produced in different countries and at different latitudes, it is worthy of note that, in general, both the free and ester menthol content, and hence the aromatic properties and value, increase proportionately as higher latitudes are reached. This point is illustrated by analyses of peppermint oils from different latitudes, chosen at random. Thus an oil from the Jordan Valley in Palestine (Israel) (ca. 33° N. Lat.) was reported to contain only 12.9% total menthol, of which 5.57% was ester; the odor was strongly reminiscent of pennyroyal and might suggest mistaken identity (26). In contrast, oils produced in Poland (ca. 54° N. Lat.) showed 48.17–68.18% total menthol, with 6.25–14.0% menthyl esters (30). On the other hand, Fahmy et al. (10) reported total menthol figures ranging from 46.32 to 58.0% and menthyl ester values from 6.82 to 15.6%, using peppermint oils produced in northern Egypt (ca. 30° N. Lat.).

The climatic and soil preferences of peppermint may best be judged from a consideration of the areas in which it is most widely cultivated. Such data indicate that the plant grows best in "continental" and modified "continental" types of climate as contrasted with "insular" climates. Large-scale cultivations are carried on profitably in the United States (especially Oregon, Washington, Michigan and Indiana), the U.S.S.R., Canada, Argentina, England, France, Italy, Poland, Germany, Roumania, Bulgaria, Hungary, Latvia and Morocco. Production in North America is thus largely conducted in the humid continental and oceanic climatic zones; that in Europe in the cool temperate, oceanic and Mediterranean types of climate, in which continental conditions are strongly modified by marine influences. In general, from what is available, it would appear that peppermint is best adapted to a fairly cool, wet spring and a warm, dry summer.

[5] It is often difficult to determine precisely which peppermint oil is referred to in commercial, industrial and statistical publications, since all too often the true peppermint, the altogether different Japanese peppermint, and the cornmint oils (the latter dementholized Japanese peppermint oil) are not distinguished.

Cultivation in the Southern United States

A few reports on peppermint cultivation in the southern United States have appeared in the literature. In **1869**, Porcher (**24**) stated that the mints (including peppermint) flourish in the Southern States: "the essence and mint water can be extracted in any quantity". In **1881** the cultivation of peppermint was said to have been introduced into the southern States, where good crops were reported, although no oil had appeared on the market. Curry (**8**) said that Tennessee was a most favorable location, and Long (**21**) claimed to have grown peppermint (and spearmint) successfully and with excellent yields at Sherman, Texas (Lat. **33.5°** N.). According to Glenk (**13**), experimental plantations on reclaimed land

TABLE I

RECORD OF PEPPERMINT CROPS AT THE MEDICINAL PLANT GARDEN, UNIVERSITY OF FLORIDA, 1927–1942, INCLUSIVE

Year	Date of Distillation	Variety of Peppermint	Fertilizer (if Known)	Area or/and No. of Rows	Wt. Green Herb, lbs.	Equiv. in lbs. Green Herb/Acre	Stand	In Charge of Medicinal Plant Garden
1927	IX-9-27	English	....	.... No data				H. W. Werner
1928	.... New stock introduced—no cutting or distillation							H. W. Werner
1929–1930	.... No data on True Peppermint							H. W. Werner
1931	IX-23-31	English (Oregon)	Not stated	0.062 acre	140	2240	....	L. D. Hiner
1932	VIII-12-32 1st cutting)	English (Oregon)	5-7-5 at 292 lbs. per acre; appl. 5-6-32	0.041 acre (6 rows)	316	7707	Good. Reset 2-23-32	L. D. Hiner
1933	....	English (Oregon)	5-7-5 at 221 lbs. per acre; appl. 4-18-33 + 5-8-33	0.136 acre (20 rows)	.. No data ..		Very poor. Plants set in field III-2-33 Up by III-23-33	L. D. Hiner
1934	Plants set out IV-10-34; up IV-24-34.			..... No data			Fair	R. S. Justice
1935 to 1939, incl.	.... No data							E. J. Ireland
1940	VIII-?-40	....	Very little *	....	...	....	....	C. H. Johnson
1941	VII-22-41	....	Nitrate of Soda—158 lbs. per acre	0.126 acre	328	2603	Fair	C. H. Johnson
1942	VII-9	....	4-7-5 at 794 lbs. per acre	0.126 acre	365	2897	....	C. H. Johnson

* Much rain in July. Plants standing in water for long period, defoliated, mostly stems distilled. Plants about 18" high.

TABLE II

Record of Peppermint Oils Produced at the Medicinal Plant Garden, University of Florida, 1929–1942, Inclusive

Year	Variety of Pepper-mint	Volume of Oil in cc.	Spec. Grav. Oil/25° C.	Weight of Oil in Gm.	% Yield on Green Herb (W/W)	Yield of Vol. Oil per Acre (lbs.)		Miscel-laneous
						Per Harvest	Per Annum	
1931	English (Oregon)	70	(0.92)	64.0	0.101	2.4	2.4	....
1932	English (Oregon)	630	0.8974	565.0	0.395	30.4	30.4	....
1933–1939 incl.	....	....	....	No data	....	....	....	....
1940	....	About 20	.....	About 20	Negli-gible	Negli-gible	....	See footnote Table I
1941	....	...	.....	342.2	0.230	5.99	5.99	....
1942	....	720	.....	(648.0)	0.39	11.35	11.35	....

near Raceland, Louisiana (29° 45′ N. Lat.), were not successful because of the poor quality of oil produced. He blamed this on contamination with odoriferous weeds which were most difficult to keep from growing with the mint. It is said that this same factor, among others, discouraged cultivation in California. The high hopes held out for the industry there about 1927 have declined, and there is said to be at present no commercial production [6].

As far as could be determined, no commercial cultivation of mint species exists at present in the State of Texas. According to Prof. C. C. Albers (Univ. of Texas):

" About 20 years ago there were about 100 acres of peppermint under cultivation in Galveston County, Texas, but I am unable at this time to obtain any information additional to newspaper accounts of the status of the farm. I am of the opinion that it is no longer in production ".

" About two years ago I attempted the growing of peppermint, spearmint and Japanese peppermint in the medicinal plant garden here at the University. There was fair growth of all three species during the first year or two, but thereafter all died. The summer months are too hot and dry for these plants to survive without much watering and care. It may be that the root rot which is very destructive to the cotton plant throughout the black land belt of Texas may have been responsible, at least in part, for the death of these mints ". (Personal communication, Jan. 15, 1947).

It has been suggested (3) that the irrigation district of the Lower Rio Grande valley might be a good location for mint cultivation.

Experimental Cultivation in Florida

The records of the Medicinal Plant Garden of the University of Florida (29° 40′ N. Lat.) included peppermint for the first time in 1927. Information on cultivations beginning that year is given in Tables I and II.

The soil in the cleared eastern area of the Garden where peppermint has been grown is for the most part Blanton Fine Sand (formerly called Alachua Fine Sand), with some admixture of other soils. A representative sample

[6] Personal communications from the office of the Director, College of Agriculture, Univ. of California, and from R. C. Baines, Indiana Agricultural Experiment Station, quoting W. W. Robbins, Davis, California.

from the peppermint-planted area showed a pH of 5.94 (fall, 1940). This soil allows fair to good natural drainage, but is considered poor for truck crops. The practice has been to replant peppermint every year or two, alternating it with other medicinal plants and with *Crotalaria* species.

1927: In the spring, English peppermint (presumably *Mentha piperita* L. *pro parte* Huds. var. *officinalis* Briquet) [7] was planted in the south end of the Garden tract just acquired; it was transplanted about July 10 because of the unusually dry season. By the time of cutting it had formed a good stand. "Roots" (runners) were recorded as "fairly good". By September 23 the plants were showing the effects of drought, but expansion of the stand was evident. American peppermint (which is said to be a form of *Mentha piperita* standing between var. *citriodora* G. F. W. Meyer (var. *citrata* (Ehrh.) Briq. and var. *calophylla* Briq.) was also grown, but not in sufficient quantity to distil.

1928: About January all mints in the Garden were winter-killed—probably in part on account of the extreme drought. New stock was obtained from the Bureau of Plant Industry (U.S.D.A., Washington, D. C.), and this was transplanted to a new location about April 1; it was allowed to spread and form plenty of rootstock during the year. This stock of "American Peppermint" from Washington, D. C., which produced "abnormal" peppermint oils (ca. 1929–1931) was later shown by one of the authors to be actually a clon of the *Mentha arvensis* complex, probably *M. canadensis* L., most commonly known as "American (Wild) Mint" (18) [8].

1931: A very dry year, especially towards fall. Root stock of English mint obtained from Oregon was planted in the late spring. The stock behaved much as did the so-called "American peppermint" from Washington, D. C., i.e., the plants were inclined to lodge (or become procumbent) and hence were difficult to harvest.

1932: This was another dry year. The Oregon peppermint proved to be low-growing and late in coming into bloom, so that only the first crop could be harvested. No records were made of the other "peppermints".

1934: An unfavorable season because of a long dry period until mid-April, followed in late April and May by heavy rainfall. Hot sun then scalded the moist vegetation. Oil distillation was not recorded.

1941: Only one cutting was made, this in late July. As usual for north Florida, there were a dry spring and damp summer, the reverse of what appear to be optimum conditions for growth of peppermint.

1942: This was the last year that peppermint was distilled here. The area was replanted on April 3, 1942, using 100 lbs. of 4–7–5 fertilizer on the 0.126 acre plot [9].

Further information on the experimental growing of peppermint in Florida is contained in letters received by the authors. Thus Mr. S. C. Hood, who did experimental work for the U. S. Department of Agriculture about 40 years ago, first at Huntington, then at Orange City and finally at Orlando, Florida,

[7] Identity of the variety and form of the Garden peppermint could not be definitely established, due to inadequacies of the literature. The classifications indicated are based on the key of Schinz and Keller, "Flora der Schweiz".

[8] The error in classification led to fallacious findings for "peppermint" oil.

[9] Prof. Carl H. Johnson (Univ. of Florida) says: "We have about given up any hope of doing anything with this oil". (Personal communication, 1947).

wrote that most of the *Mentha* species he dealt with "seemed to suffer from the heat of summer or from sun scald during the rainy season". Mr. W. A. Willson, formerly associated with the Penney-Gwynn Corporation, described the results of experimental growing of peppermint in the vicinity of Penney Farms, Florida, as follows:

"From a commercial standpoint, it was not a success. The plants grew well enough but the stems were so weak and spindly that they would not stand up during our summer rainy season and as a result the whole patch flattened to the ground and was beaten into the sand by the hard showers. We planted the mint on one of the richest plots of land we had but the stems did not grow strong enough to withstand the rains". (Letter of Nov. 5, 1941).

Peppermint has also been grown experimentally at various times in the Everglades. The United States Sugar Corporation at Clewiston, Florida, cultivated peppermint (using stock from the Medicinal Plant Garden of the University of Florida) in the biennium 1935–6. Five plots of 1/300 acre each were used, and the average of all (totalling 1/60 acre) taken. The field data are presented in the table below:

Date	Yields per Acre		Yields Percent of Green Herb
	Green Herb	Volatile Oil (lbs.)	
VII-22-35, Planted			
VII-7-36, Harvest I	10.66 tons; 21,320 lbs.	33.96	0.159%
X-15-36, Harvest II	6.56 tons; 13,120 lbs.	20.16	0.154

Subsequent to harvesting, the stubbles [bases of plants] died out. This suggested that one crop annually might be better practice for this area. The soil was an "intermediate mineral muck type, commonly called willow-elder soil". Fertilizer was applied in amounts per acre of 200 lbs of muriate of potash, 60% K_2O; 200 lbs. superphosphate, 19% P_2O_5; 50 lbs. $CuSO_4 \cdot 7\,H_2O$; 25 lbs. $MnSO_4$. In this early experimental work the plants did not appear to lodge excessively. Analysis of the volatile oils obtained showed a strikingly inferior oil, reminiscent in composition and constants of that reported from plants at the University of Florida (18):

Characteristic	Clewiston Oils *	Usual Commercial Oils
Sp. gr./15° C.	0.9123	0.910–0.920
Soly. in 70% alc.	1 in 2 vols.	1 in 2 to 5 vols.
Acid number	0.60	Less than 1
Ester (as menthyl acetate)	6.01%	6 –10%
Combined menthol	4.74	5 – 9
Free menthol	28.66	43–54
Total menthol	33.40	48–63

* Determinations made by M. S. Lowman, U.S.D.A.

Dr. B. A. Bourne stated that: "at the present time no commercial plantings of peppermint or spearmint exist in the Everglades region. The only commercial essential oil still in the Everglades is the one we built at Clewiston"[10].

It is obvious that the Everglades plantations of mints were not successful.

Yield of Herbage

Peppermint herb as such is used to a limited extent in this country, and the largest if actually not the entire amount is obtained from wild plants in North Carolina. The herbage productions from cultivated areas in the U.S.A. seem to be used entirely for volatile oil production, since the economic returns are so much higher from the oil. In Europe, on the other hand, the herb is considered of outstanding importance for

[10] Personal commun. from B. A. Bourne, Chief of Agr. Research, now Vice President, U. S. Sugar Corporation, Clewiston, Fla., Sept. 29 and Nov. 19, 1941; Jan. 9, 1948; Jan. 8, 1951.

the preparation of infusions ("teas"), &c., and a large share of the cultivated peppermint produced there is used in preparing the crude drug "Herba (or Folia) Menthae piperitae". In consequence, nearly all reports of any accuracy on herbage yield per unit area originate from Europe, and little information on this point can be gleaned from the American literature. According to one estimate, the American yield is two to three tons per acre (2). If this estimate is substantially correct, the Florida Garden yields for the single year 1932 may be regarded as above average, with an acre equivalent of 7,707 lbs. (3.85 tons). However, the yields reported for other years were unusually poor (1.12 tons of English peppermint in 1931; 1.30 tons in 1941; 1.45 tons in 1942), perhaps on account of the customary unfavorable growing weather.

Yield of Volatile Oil

Reported yields of peppermint oil in the United States vary from almost nothing to 153 lbs. avoir. per acre per year (31) [11]. It will be noted that yields of volatile oil from the University of Florida Medicinal Plant Garden (Table II) show considerable variation from year to year; the average yield per acre ranged from a low of 2.4 lbs. in 1931 to as high as 30.4 lbs. in 1932.

The average yield in the four years for which we have records is 12.53 lbs. per acre per year. This is considerably inferior to the 30 lbs. per acre frequently mentioned as representing an average yield [12], and very much below the yields claimed for peppermint on the Pacific coast, where 40–50 lbs. (20) to 60 lbs. (19) per acre per year is usual, and where 80 to 100 lbs. yields are not uncommon (9) [13].

Quality of Volatile Oil

The following features, among others, are generally recognized as indicating good quality in peppermint oil for medicinal and food usage: (*a*) high menthol content; (*b*) high menthol ester content, giving aromatic odor and taste; (*c*) low content of menthone (said to give a bitter after-taste (27)) and other ketones. A better idea of the qualities of a good oil may be had from the analyses of oils produced in the States of Washington and Oregon, and universally acknowledged as of good quality [14]. Such oils were studied by Johnson and Wilkes (19) who reported constants ranging as follows: total menthol, 59.99 to 78.45%; combined menthol (calculated as menthyl acetate), 7.90 to 20.46% (averaging about 10%). Other studies of these western oils were made by Tornow and Fischer (32) who found that total menthol ran 36.63 to 72.07% (average 55.18%) and combined menthol 2.9 to 16.26% (average 6.72%).

The composition of a number of the oils produced from the University of Florida Garden plants is shown in Table III. It will be noted that assays of oils obtained from the English mints of Oregon ran below requirements of the United States Pharmacopeia (not less than 50% total menthol), perhaps because of environmental factors.

Pot Experiments in Greenhouse

Runners of peppermint from the Medicinal Plant Garden were trans-

[11] It is more accurate to give yield in pounds per acre (or an equivalent) than in proportion to herb weight, since the water content of the latter varies so greatly; yields are also best stated as annual totals to indicate actual productivity.

[12] For example, by Horace Finnemore (11); however, his data represent yields from only one crop per year, while the Florida figures are totals for several crops per year.

[13] Yields in Washington for 1930–1952 (incl.) averaged about 47.3 lbs./acre; for Oregon for 1930–1952 (incl.) about 40.6 lbs./acre (34).

[14] With the possible exception of the Kennewick (Type K) oils of eastern Washington (32).

planted in glazed earthenware crocks containing various soils of Florida, previously dried at room temperature and screened through a five-mm. sieve. Unless otherwise noted, crocks of one gallon capacity were used. Watering was carried on three times weekly, an attempt being made to add water only to the point of saturation, as indicated by run-off from the bottom drainage tube. This drainage was collected in jars and returned to the soil at the next watering. This was done in order not to leach out and lose the salts normally present in the soil, and thus to more nearly approach conditions in the soil in situ. Distilled water (pH 4.5 to 5.0 to Nitrazine paper) was used until May 20, 1941; after that, tap water (pH approx. 7.5 to Cresol Red). Soil moisture was determined by weighing the soil first in the air-dried state and again at the end of the experiment, which was always either two or three days after the last watering, so that the percentage given may be regarded as minimum and perhaps more fairly representative of the usual condition of humidity. Fertilizer applications were made on the basis of weight (acre-furrow-slice of 2,000,000 lbs. for inorganic soils, 500,000 lbs. for "Saw Grass" Peat, and 600,000 lbs. for "Custard Apple" Muck) in all but two cases where area was used as a basis.

TABLE III

Constitution of Peppermint Oils from Medicinal Plant Garden, University of Florida (1929–1932)

Date Oil Produced	Det'd. by: *	Free Menthol	Ester Menthol †	Total Menthol	Other
IX-23-31 English (Ore.)	A	(36.61)	7.03	43.64	....
VIII-12-32 English (Ore.)	A	(32.27)	6.47	38.74	Considerable amounts menthone
	B	33.44	8.63	42.07	
	C	26.08	....	....	3.0% pulegone ‡
VII-9-42	Student	32.63	11.87	44.50	....

* Key to symbols used in second column:

A—Lovell D. Hiner, "Mint Oils in Florida", Unpublished M.S. in Pharm. Thesis, University of Florida (1931); and garden records. Used U.S.P. X method; assays run 1929–30.

B—Arnold Whitney Matthews, "A chemical investigation of some Florida volatile oils", Unpublished Ph.D. Dissertation, University of Florida (1941). Used U.S.P. XI method; assays run 1940–1.

C—Arnold Whitney Matthews, *loc. cit.* Used Brignall method (*Industrial and Engineering Chem., Analytical Edition,* Vol. 13 (1941), p. 166); assays run 1940–1.

† Calculated as menthyl acetate.

‡ Determined by neutral sulfite method (E. Gildemeister und Fr. Hoffmann, *Die Aetherischen Oele.* 3. Aufl. (1931)).

The herbage was carefully dried to air dryness for five to seven days after collection, and the volatile oil obtained by distillation in a Clevenger apparatus. Oil yields were estimated on the basis of both fresh and dried herbage. Water content of herbage was estimated from the difference in weight of the fresh and air-dried materials.

As criteria of the state of vegetative development, habit and height of plant and dimensions of stem and leaf were

TABLE IV

RESULTS OF EXPERIMENTAL GREENHOUSE CULTIVATION OF PEPPERMINT IN VARIOUS SOILS OF FLORIDA FOR 98–102 DAYS

Reference Number of Culture	Soil	Number of Days Grown	Fertilizer	Wt. Gms. Green Herbage	Wt. Gms. Air-dried Herb	Herb Moisture %	Soil Moisture %
1	Leon Fine Sand	98	None	2	Insufficient to determine		...
2	Same	98	None	Dead	...	...	...
3	Same	98	Lime Sulf. Pot.	36	15.3	74.9	17.2 (aver. 18.4)
4	Same (2 gal.)	98	Superphosph.	25			19.5
5	Fellowship	98	None	47	7.6	83.8	13.6
6	Fine Sandy	98	None	38	6.4	83.2	...
7	Loam	98	0–12–24 at 500 lbs.	66	29.5	78.2	9.8 (aver. 10.1)
8	Same	98	per acre	69			10.4
9	Bayboro Fine Sandy	98	0–12–24 at 500 lbs.	32	8.4	87.5	21.6
10	Loam	98	per acre	35			...
11	Arredondo Fine Loamy	99	Lime, Mur. Pot., Nit. Soda, Superphosp.	43	7.6	91.2	10.6
12	Sand	..		43			
13	Same (5 gal.)	99	Ammophoska, 1000 lbs. per acre *	134	23.2	82.7	...
14	Custard Apple	98	None	46	6.5	91.8	11.3 (aver. 12.6)
15	Okeechobee Muck	98	None	33			13.8
16	Same	98	0–12–24 at 1000 lbs.	51	16.8	84.1	16.8 (aver. 15.8)
17	Same	98	per acre	55			14.8
18	Same	98	0–12–24 at 500 lbs.	47	16.4	78.1	17.3 (aver. 19.0)
19	Same	98	per acre	28			20.6
20	Saw Grass Everglades	98	None	59	Plants replanted and grown for a total of 185 days (see Table V)		
21	Peat	98	None	45			
22	Same	98	0–12–24 at 500 lbs.	19	6.0	89.3	20.1 (aver. 26.5)
23	Same	98	per acre	37			32.8
24	Same	98	Tobacco ash	15	2.2	85.3	29.1

* Figured on area basis (5 gal. crock = 0.000014 acre).

determined for each culture [15]. The hydrogen ion concentrations of the air-dried soils were determined at the finish of the experiment. The method used was that followed at the Florida Agricultural Experiment Station (7) a Beckmann hydrogen-ion electrometric apparatus being used.

[15] Incidentally, none of the greenhouse peppermints flowered, although they normally do so in the field in Florida. No doubt this resulted from the reduction in insolation brought about by a thick coat of whitewash applied to the sides and top of the greenhouse. Nonflowering is often noted in England, and possibly also this results from the well-known deficiency of sunlight there. Scotch spearmint (*Mentha cardiaca*) and Japanese mint growing in the greenhouse blossomed normally.

TABLE IV (*Continued*)

Reference Number of Culture	Soil	Habit of Plant	Root System Extent	Stem (Max.) Height cm. 6-4-41	Maximum Lamina Dimensions in mm.		Soil pH at End of Growth
					Length	Breadth	
1	Leon Fine Sand	Weak and degenerating	....	7	..	..	...
2	Same	Very small; dead	....	12	..	..	...
3	Same	Average	....	21	45	24	4.14
4	Same (2 gal.)	Average	....	15	34	18	
5	Fellowship	Average	Rather	42	54	28	5.13
6	Fine Sandy Loam	Average	extensive	37	41	23	
7	Same	Average	Strongly developed	56	46	26	5.24
8	Same	Average	Very extensive	40	45	25	
9	Bayboro	Straggling	Small	50	40	20	4.79
10	Fine Sandy Loam	"	"	40	47	24	
11	Arredondo	Average	Rather	7	35	21	6.39
12	Fine Loam Sand	Average	extensive	10	29	16	
13	Same (5 gal.)	Average	Same	12	54	31	6.24
14	Custard Apple	Sprawling	Extensive	37	48	26	5.57
15	Okeechobee	"	"	47	44	22	
16	Muck	"	"	45	59	31	...
17	Same	"	"	36	55	31	...
18	Same	"	"	52	53	27	5.57
19	Same	"	"	32	32	17	
20	Saw Grass	Average	....	45	54	28	5.43
21	Everglades Peat	"	....	40	54	26	
22	Same	"	....	22	30	16	5.53
23	Same	"	....	30	46	22	
24	Same	"	....	30	42	21	...

Soils and Fertilizers

In some cases growth was tried in both unfertilized and fertilized soils of the same type; in other cases, the fertilized soil alone was used. In every instance two pot cultures were employed for each formula. In determining herb moisture and in distilling the oil, the herb samples were combined; in determining soil moisture, the average of the two figures was used; and in finding the soil pH, the soils were admixed to secure a representative sample:

1) Leon Fine Sand: Collected 12/19/40 from saw palmetto tract near Payne's Prairie, using the first five inches from surface. This soil is considered poor for truck crops (17). Fertilizer was applied at the acre rate of 2000 lbs. ground limestone, 150 lbs. potassium sulfate, and 600 lbs. Superphosphate.

2) Norfolk Fine Sand: Obtained 10/27/40 from turkey oak scrub land near

TABLE IV (*Continued*)

Reference Number of Culture	Soil	Maximum Stem Thickness (mm.)	Volatile Oil Yield 0.01 cc.	Vol. Oil Yield % V/W Green Herb	Vol. Oil Yield % V/W Dried Herb	Miscellaneous
1	Leon Fine Sand	..		Not determined because of insufficient herb		
2	Same	..				
3	Same	2.7	16	0.19	1.14	....
4	Same (2 gal.)	2.1				
5	Fellowship Fine	3.0	16	0.19	1.14	....
6	Sandy Loam	2.1				
7	Same	3.7	26	0.18	0.88	....
8	Same	4.8				
9	Bayboro Fine	2.3	6	0.09	0.71	....
10	Sandy Loam	2.2				
11	Arredondo	2.5	10	0.12	1.32	The large yield of #13 may be due to better development in large pot
12	Fine Loamy	2.3				
13	Sand (5 gal.)	4.1	29	0.22	1.25	
14	Custard Apple	3.0	17	0.22	2.61	....
15	Okeechobee Muck	2.3				
16	Same	3.1	13	0.12	0.77	....
17	Same	2.9				
18	Same	2.8	11	0.15	0.67	....
19	Same	3.1				
20	Saw Grass Ever-	3.6		Not distilled until after total of 185 days' growth (See Table V)		
21	glades Peat	3.0				
22	Same	2.6	8	0.14	1.33	....
23	Same	2.6				
24	Same	2.1		Too small an amount to distil		

Gainesville, off the Palatka road. Said to be unsuited for truck crops (Henderson). Applied fertilizer as follows: potassium sulfate, 150 lbs.; sodium nitrate, 150 lbs.; Superphosphate, 600 lbs. per acre.

3) Fellowship Fine Sandy Loam: Collected 12/19/40 from slightly hilly land near Gainesville, using the top six inches. Fertilizer of the formula 0–12–24 was applied at the rate of 500 lbs. per acre [16].

4) Bayboro Fine Sandy Loam: Collected 10/27/40 from field close to Alachua Sink, Payne's Prairie, near Gainesville, using the first six inches. Said to be good for truck crops (Henderson). Fertilized with 0–12–24 at 500 lbs. per acre.

5) Arredondo Fine Loamy Sand: Collected 3/28/41 near Gainesville. pH at time of collection was 6.01. Two cultures (Nos. 11, 12) were fertilized with the equivalent of 1000 lbs. ground limestone, 150 lbs. sodium nitrate, 150 lbs. potassium chloride, and 600 lbs. Superphosphate to the acre. Another culture (No. 13) in a five-gallon crock (= 0.000014 acre) received on an area basis the equivalent of 1000 lbs. Amophoska per acre.

6) Orange Lake Peat: Collected about March, 1941, from W. A. Shand's farm, close to Orange Lake, Florida. pH 4.87 at time of collection. On basis of area (1 gallon crock = 0.0000059 acre), used the equivalent of the following: ground

[16] This fertilizer formula and those which follow are given in the order: nitrogen–phosphate–potash.

TABLE V

Results of Greenhouse Cultivation of Peppermint in Various Soils of Florida for Period of 175–187 Days

Reference Number of Culture	Soil	Number of Days Grown	Fertilizer	Wt. Gms. Green Herbage	Wt. Gms. Air-dried Herbage	Herb Moisture %	Soil Moisture %	Soil pH End Growth	Habit of Plant
25	Norfolk Fine Sand	175	Sulf. Pot., Nitrate Soda, Superphosph.	69	7.9	88.6	17.9	5.37 *	Most erect and sturdiest of all
26	Saw Grass	185	None	184	45.4	75.3	12.8	5.82	......
27	Everglades Peat	185 †	None	347	90.9	73.8	38.3 †	6.13	......
28	Orange Lake	187	Grd. Lime, Sulf. Pot.,	167 ‡	20.0	88.0	53.4	5.14	......
29	Peat	187	Superphosph.‖	206 §	...	...	...	5.18	......

* Before cultivation, soil had pH 5.85.

† This culture kept in constant contact with water (in reservoir) during the last 87 days of growth (viz., July 8, 1941, to Oct. 3, 1941).

‡ Weight of green herb after 102 days of growth 91 gm.

§ Weight after 102 days growth 82 gm.

‖ Figured on area basis (1 gal. crock with surface area of 0.0000059 acre).

limestone, 2000 lbs.; potassium sulfate, 200 lbs.; Superphosphate, 600 lbs. to the acre.

7) "Saw Grass" Everglades Peat: Collected during November, 1941, at Belle Glade, Fla. Applied 0–12–24 at 500 lbs. per acre to cultures No. 22 and 23. Culture No. 24 received tobacco ash at the rate of 504 lbs. per acre.

8) "Custard Apple" Okeechobee Muck: Collected November, 1940, at Lake Harbor, Fla. Used fertilizer mixture 0–12–24 at the rate of 500 lbs. per acre on one set, at rate of 1000 lbs. on another.

Results from these pot experiments are recorded in Tables IV and V.

Discussion

Field experiments at the University of Florida Medicinal Plant Garden, Gainesville, Florida, conducted between 1927 and 1942, indicate that the growth of peppermint in this region is erratic throughout the year and also from year to year. Although development appears excellent as a rule during the spring months, the plants usually develop a poor stand later and are inclined to lodge; this makes harvesting difficult [17]. Although herbage may seem fairly normal in appearance and amount, especially early in the year, it generally shows at the most only an average yield of volatile oil, and this of relatively poor quality. It might prove profitable in southern latitudes to distil the herb as early in the year as possible—June or even earlier. This would require early transplanting in years when carried out. Cutting in late spring would permit the herb to vegetate during a period of adequate moisture and to mature in the drier days of early fall. However, only the mature flowering plant gives a good quality of oil, as has been shown fre-

[17] The difficulty in effectuating a proper degree of stubbling has been reported as the chief obstacle in economic production of peppermint and spearmint in the Everglades area (personal communication, Dr. B. A. Bourne, U. S. Sugar Corporation, Jan. 9, 1948).

TABLE V (*Continued*)

Reference Number of Culture	Soil	Number of Days Grown	Root System Extent	Maximum Stem: Height cm. 6-4-41	Maximum Stem: Thickness mm.	Maximum Lamina: Length mm.	Maximum Lamina: Breadth mm.	Volatile Oil Yield: 0.01 cc.	Volatile Oil Yield: % (V/W) Greenage	Volatile Oil Yield: % (V/W) Air-dry Herb
25	Norfolk Fine Sand	175	Very restricted	47	4	52	24	3	0.04	0.38
26	Saw Grass	185	....	45	3.4	26	18	9	0.05	0.20
27	Everglades Peat	185 *	....	40	3.3	35	20	14 †	0.04 †	0.15 *
28	Orange Lake	187	Fairly extensive	45	3.6 ‡	53 ‡	30 ‡	10	0.06	0.50
29	Peat	187	Same	42	3.8 ‡	36 ‡	18 ‡	..	...	...

* This culture kept in constant contact with water (in reservoir) during the last 87 days of growth (July 8 to Oct. 3, 1941).

† Dissimilarly from ordinary procedure, in this case the distillation was carried on with only partially dried herb.

‡ Data for these cultures after 102 days growth:
No. 28—Max. thickness of stem 3.6 mm.
Max. leaf dimensions: 20 × 42 mm.
No. 29—Max. thickness of stem 3.5 mm.
Max. leaf dimensions: 27 × 36 mm.

quently. Thus Fahmy et al. (10) found for plants grown in the Pyramid area of Egypt that total menthol and ester percentage (resp.) ran for pre-flowering plants, 47.0–50.2% and 9.1–11.0%; for partly flowering plants, 57.5% and 13.5%; for fully flowering plants, 56.8% and 15.6%.

Another possible procedure might be to set the plants out during the rainy season (about June) and to harvest them in autumn. This is said to be the practice with mints and other herbs in tropical areas of Mexico. Shading would no doubt prove advantageous during the summer months.

In the greenhouse experiments, growth under identical conditions proved quite variable for different soils used. The latter might be arranged with reference to various characteristics of the mints growing in them—e.g., oil yield, herb growth, etc. An effort was made to find relationships between such characteristics of the soil as moisture and pH, on the one hand, and measurable characteristics of the cultured plants, such as leaf size [18] and oil content, on the other. However, no very definite correlation could be adduced from the various data, as might be expected from the complication of factors involved. Since the volatile oil produced is the item of prime interest, it was thought most logical to arrange the various soils in the order of total oil production from plants growing in them, and this has been done (Table VI).

The importance of abundant moisture in the development of both herbage and volatile oil is suggested by the results obtained in the 185-day culture of peppermint in "Saw Grass" Everglades

[18] The importance of leaf dimensions, etc., in such a study as this was well shown in a paper by Bode (4).

Peat (unfertilized). The culture was so conducted that the soil was in continuous contact with water [19]. During the last 87 days of growth, the plant showed a green herbage weight 188%, an air-dried weight 200%, and an oil production 156% of that of the culture watered in the usual way.

That species of *Mentha* are best adapted for relatively humid soils and somewhat shaded or cloudy localities may be deduced from the structural characteristics of these plants, such as the typical mesophytic leaves, the thin cuticle on leaves of this type (33) and the stomata somewhat raised above the epidermal surface (14). The practical experience of growers in various parts of the United States and the greater success of plantations in the temperate Pacific Coast States have already been discussed.

Data on field-scale cultivations of peppermint in Florida are not abundant. Many rumors of growing the plant in Florida are heard. When investigated, however, these have almost always proved to be false [20]. Many people have no doubt attempted cultivation in this State from time to time over a long period. This is indicated by the many inquiries about peppermint received by the College of Pharmacy of the University of Florida and by the Florida Agricultural Experiment Station. It appears evident that if this plant could be grown as successfully as or more successfully than in other States, there would now be a considerable acreage of it in the State, particularly in view of the advantages cited in the next paragraph and because of the generally favorable price of the oil. That such enterprises do not exist is fairly good evidence that attempts at profitable cultivation in Florida have failed. Furthermore, in this region *Mentha* species appear to be as uncommon in the wild state as in the cultivated. In four State-wide surveys of native wild life, R. M. Harper and others (16) reported that no members of this genus were common in Florida.

There are several advantages in growing peppermint in Florida: (*a*) relative freedom from many of the pests found so detrimental in the north (e.g., in Indiana), especially mint flea beetle and certain fungous diseases (such as rust and

TABLE VI

PEPPERMINT OIL YIELD FROM PLANTS GROWING IN VARIOUS SOILS OF FLORIDA IN GREENHOUSE CULTURES

(Arranged in descending order of total oil yields)

Soil and Treatment	Culture Numbers	Oil Yield 0.01 cc.
After growth period of 98 to 102 days		
Fellowship (fertilized)	7, 8	26
Arredondo (fertilized with Ammophoska) *	13	24 †
Custard apple (unfertilized)	14, 15	17
Fellowship (unfertilized) ...	5, 6	16
Custard apple (fertilized at 1000 lb. rate	16, 17	13
Leon (fertilized)	3, 4	12 †
Custard apple (fertilized at 500 lb. rate	18, 19	11
Arredondo (limed and fertilized)	11, 12	10
Saw grass (fertilized)	22, 23	8
Bayboro (fertilized)	9, 10	6
After growth period of 175 to 187 days		
Saw grass (Water Reservoir) (unfertilized)	27	14
Orange Lake peat (fertilized)	28	10
Saw grass (unfertilized)	26	9
Norfolk (fertilized) (1 culture)	25	3

* Single culture in large crock (5 gallon) probably partly responsible for more vigorous growth and larger oil production.

† Corrected (by areas) to make allowance for crocks of different size.

[19] For this purpose the potted plant was set into a larger crock kept filled to a certain level with tap water.

[20] For instance, the newspaper *Homestead Enterprise* in 1928 stated (apparently without foundation) that peppermint could be grown thereabouts successfully.

anthracnose); these parasitic organisms do not seem to prevail here; (*b*) possibility of two or three harvests in good growing seasons; (*c*) almost no risk of freezing the stolons in the winter; this is a frequent cause of crop failure with mint in Michigan and Indiana; (*d*) a cheaper labor market.

Disadvantages in growing peppermint in Florida include: (*a*) frequence of lodging or development of procumbency by the plants; this makes harvesting much more difficult, if not practically impossible with modern equipment; (*b*) to offset lodging it has been found wise to replant each year, since by so doing, growth seems hardier and more erect, but this increases labor costs, whereas in the northern and western fields, replanting is not ordinarily done for intervals of several years; (*c*) even when they have been replanted in successive years, the plants tend to lodge more and more, and the oil yield of successive crops diminishes; (*d*) apparently greater shattering (or leaf fall) occurs in Florida plantings; this may result from the rainy weather of the summer months, as has been observed in other areas (**6**); (*e*) oil quality has on the whole proven poor; (*f*) weeds grow more prolifically here than in the north and weeding is more difficult because of the procumbent habit of the mint, adding to the labor costs; (*g*) long distances and hence higher shipping costs to areas of consumption are other important disadvantages.

Conclusions

From all the facts available, cultivation of peppermint in Florida does not appear to be practicable or commercially profitable.

Among the eight soils studied in greenhouse cultivations, peppermint apparently grows to best advantage in Fellowship Fine Sandy Loam, "Custard Apple" Everglades Muck, and "Saw Grass" Everglades Peat, in descending order.

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Utilization Abstracts

American Desert Plants. Two hundred fifty thousand square miles of hot desert land in the southwestern United States, and 400,000 square miles of similar territory in the adjoining northern part of Mexico support a considerable variety of plants which have commercial potentialities, but only a few of which have been utilized. The latter are several species of *Pedilanthus* and *Euphorbia* which furnish candelilla wax; beargrass (*Nolina microcarpa*) which provides an excellent substitute for broom straw; several kinds of *Agave* that yield valuable fiber and serve in manufacturing the important Mexican alcoholic beverages mezcal and tequila; creosote bush (*Larrea divaricata*), the source of nordihydroguaiaretic acid, a valuable antioxidant; canaigre (*Rumex hymenosepalus*), a potential source of tannin; various species of *Yucca* to furnish fiber; and guayule (*Parthenium argentatum*) to furnish rubber.

Promising but less advanced with respect to potential utilization are jojoba (*Simmondsia chinensis*) as a source of wax; various wild gourds (*Cucurbita* spp.) to supply oil and protein; devil's claw (*Martynia parviflora*), also to furnish edible oil and protein; and a number of other plants. (P. C. Duisberg, *Desert Research. 1953*).

Tannins and Resins in Drilling Muds. In petroleum engineering fresh water and brine muds with various additives are used to line the drill holes with an impervious coating so as to prevent seepage of water and to accomplish other technological objectives. The additives include various chemicals, starch, tannin extract of chestnut and quebracho wood and various gums. Among the latter, either actually used to some degree or experimented with for the purpose, have been tragacanth (from various species of small thorny shrubs in the genus *Astragalus*), karaya (from trees of *Sterculia urens*), ghatti (from several trees of *Anogeissus*), gum shiraz (the botanical source of which seems not to be definitely known), gum arabic (from various species of *Acacia*) and the gums of locust bean (*Ceratonia Siliqua*) and quince seed (*Cydonia oblonga*).

BOOK REVIEWS

Vegetable Tanning Materials. F. N. Howes. xii + 325 pages. 1953. London: Butterworths Publications, Ltd.; American Distributors: The Chronica Botanica Co., Waltham, Mass. $5.50.

One industry which is very much aware of a threatening shortage in the supply of its vegetable raw material is that of tanning, and efforts are being made in various quarters to find and, more important, to develop on an economically profitable basis, additional sources of vegetable tannins. Publication of this book, therefore, was especially timely, for it considers over 500 kinds of plants that have been utilized in this direction, some of them many centuries. Only two or three dozen kinds have been industrially important, and in recent years the United States has depended upon imports for about two-thirds of its needs. These needs amount to approximately 120,000 tons of 100% tannin, annually, and quebracho wood of the Argentine has in recent years been the mainstay of the industry. For various reasons that source is gradually becoming more and more curtailed, and attention is being directed toward increased use of domestic oak barks, sumac, canaigre and other possible sources.

Tannins are wide-spread throughout the Plant Kingdom, and to be found in many kinds of plants which have not yet been used as commercial sources of them. Two hundred or more such plants possessing ten percent or more tannin, and thus being potential sources of tannin, are separately listed in the book. As a result of changed economic conditions, of plant breeding and of finding high-yielding tannin strains among these unexploited plants, there is always the possibility that a significant new source may be revealed.

Vegetable Fats and Oils. E. W. Eckey. x + 836 pages. Reinhold Publishing Corp. 1954. $16.50.

Some years ago, in at least two editions, Monograph #58 of the American Chemical Society was published under the title "Vegetable Fats and Oils" and under the authorship of Dr. George S. Jamieson of the U. S. Department of Agriculture. That volume, now out of print, has been succeeded by the present one as Monograph #123 and authored by E. W. Eckey of the E. W. Eckey Research Laboratory, Cincinnati, Ohio. This recent and greatly enlarged edition has retained the principal feature of its predecessor, namely, that of providing "a descriptive catalogue of nearly all of the vegetable fats and oils concerning which much scientific information has been published".

This unqualified declaration of objective, and the simple title itself, are misleading to the extent that they give the impression that at least some information on *all* important vegetable oils is contained in the book. This is far from reality, for the enormous field of essential oils, on which a six-volume encyclopedia was recently published, is scarcely mentioned. More accurately, the book is concerned only with "fixed", "non-essential" or "non-volatile" oils, so far as vegetable oils are concerned, and there never was any intention of considering the other extensive group. The book was written as a part of chemical literature, and to a chemist the expression "fats and oils" very likely always excludes essential oils, but to the present reviewer, a botanist, it appears unfortunate that the biologist or general reader consulting the book, but unacquainted with this fact, is not informed by some qualification on the title page that the oils mentioned in it constitute only one of two groups of economically important oils obtained from plants and of enormous industrial value.

With this understanding, however, the reader will find Dr. Eckey's book an outstanding compilation of information and of literature citations on the subject. As compared with Dr. Jamieson's previous volume, this one has been completely re-written, and the arrangement of material differs primarily in its not being according to the purely arbi-

trary and at times absurd basis of its predecessor, namely, that of dividing fats and fixed oils into " non-drying ", " semi-drying " and " drying " categories; instead, the fats and oils are grouped according to the natural affinities of the plants which furnish them, that is, into plant Orders, Families and larger groups.

After 240 pages in seven chapters of general chemical, physical and other aspects of these plant products, 550 pages are devoted to the individual fats and oils, beginning with the non-flowering plants and progressing through the gymnosperms and monocotyledons to the Campanulales as the highest Order of dicotyledons which furnish commercially important fixed oils. Less than a score of these plants account for more than nine-tenths of the total tonnage of vegetable fat used in the world. Most important has been coconut oil, nearly six billion pounds of which was the estimated average annual production, principally in Indonesia, Philippines, India, Burma, Ceylon, Malaya and Oceania, from 1934 through 1938. Peanut oil was a close second, followed by cottonseed, soybean, rape and mustard. Others in descending order of importance have been the oils of flax, olive, palm fruits, sesame, sunflower, palm kernels, castor, hemp, tung and babassu, the last and least important of these coming from Brazil to the extent of about 26,000,000 pounds. Of these the edible oils (cottonseed, peanut, soybean, sunflower, olive, sesame) were produced to the extent of about 18,000,000 pounds in 1951, the industrial oils (palm, flax, castor, rape, oiticaca, tung, perilla) to about 14,000,000 pounds.

Plant Regulators in Agriculture. H. B. Tukey, Editor. x + 269 pages. John Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London. 1954. $5.50.

Auxins and Plant Growth. A. Carl Leopold. xii + 354 pages. Univ. of California Press. 1955. $5.

These two books deal with precisely the same topic, namely, those substances, either produced by plants themselves or commercially synthesized and then applied to plants, which regulate cell elongation and which, because of this effect, have, in the past two decades, been the subjects of one of the most significant developments in applied botany and chemistry. These substances are known by a variety of names, two of which are " auxins " and " plant regulators ", and, in the words of one of the books, they " promise to have an impact on agriculture as great as the advent of the windmill or perhaps even of the mechanical harvester ".

The books differ primarily in the nature of their authorship, in their arrangement of material and in the relative emphasis given to matters of pure science and to those of application. With respect to authorship, the first is the work of 17 contributors, one or two of whom were responsible for each of its 16 chapters, the second is the product of one author. Regarding arrangement of material, the first book, after three chapters concerned with an introduction, with the principles of plant growth and with the chemical nature of plant regulators, devotes each of a dozen chapters to a particular aspect of application and concludes with a chapter on equipment and methods of application; the other book is divided into two large parts of several chapters each, the first part dealing with " Fundamentals of Auxin Action ", the second with "Auxins in Agriculture ". Concerning the third point of comparison, the second volume is somewhat the more technical in fulfilment of the author's intent to provide a treatise " written for the graduate student and the professional research man "; the first book is intended for " advanced high-school students, college students, and a considerable sprinkling from the business and professional world ".

The practical importance of our relatively new fund of knowledge concerning the field covered by these volumes lies in the fact that since 1934, when indoleacetic acid was first definitely identified as one of the growth-regulating substances produced within plants, this same acid, commercially produced, as well as chemically related acids, have been found effective in regulating a variety of developments in fruit trees and other plants. Although this acid has been definitely identified in only two plant sources (yeast and corn seedlings) and by indirect evidence has been shown to be the major growth hormone in the organs of six other plants (bean stems, sugar cane stems, potato tubers, oat coleop-

tiles, pineapple leaves, tomato stems), it is regarded as the most common, but certainly not the only, growth hormone in plants. Others have been identified as indolepyruvic acid, cis-cinnamic acid, phenylacetic acid and indoleacetonitrile.

While three or four dozen chemical compounds, mostly organic acids, have been found effective as growth regulators, only a few of them have acquired wide use. The most important of their practical applications, extensively discussed in these two books, may be summarized as follows:

a. To stimulate rooting in a great variety of cuttings.

b. To induce parthenocarpy in the food plants, fig and greenhouse tomato, and in the ornamental plants, holly and Christmas cherry.

c. To thin excessively heavy quantities of flowers on fruit trees, especially apples and pears.

d. To control pre-harvest fruit drop, especially in apples; oranges, lemons, grapefruit, apricot and Bartlett pear have also responded to this treatment.

e. To induce flowering in pineapple.

f. To prevent sprouting of potatoes in storage.

g. To control weeds, the greatest use of auxins, for which 60,000,000 pounds of 2,4-D were produced in the United States in 1952, from which it may be estimated that approximately 50,000,000 acres were treated with this herbicidal material.

h. To control untimely bolting and flowering in cabbage, celery and lettuce.

i. To defoliate cotton plants, preparatory for mechanical harvesting.

Each of these and several minor uses of growth-regulating substances are considered in these two books. From a long-range point of view, the chemicals concerned have great agricultural potentialities by way of overcoming certain limitations of growth and development, as in the control of fruit set and fruit drop; by way of controlling the type of growth, as in root induction; and by way of facilitating the mechanization of agriculture, as in defoliating cotton. But each of these uses is liable to create new agricultural problems by their side effects, as in producing pineapple peduncles too long and too thin to support the fruits which are induced by them to develop.